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Understanding gait analysis: Variability of Data Collected with a Pressure Sensitive Walkway

A Thesis Submitted by

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Abstract

This research is focused on two aspects of pressure sensitive walkways. The first being the general calibration process, which was evaluated in a pilot study. The second being a study of reliability (repeatability and reproducibility) of two manufacturer recommended calibration protocols assessing canine gait.

Force plates are considered to be the gold standard method of kinetic gait analysis. Although pressure sensitive walkways produce comparable results to force plates, there is great variability in the results reported amongst pressure sensitive walkway studies. Several factors may lead to the aforementioned variability, which are also common in force plate studies. One of most important, and perhaps least evaluated, is calibration methodology. Calibration methodology of a pressure sensitive walkway is more complicated than the calibration of a force plate and has indeed been less well evaluated.

Different calibration methods have led to variability in results in previous pressure sensitive walkway studies. It has been suggested that calibration *weight* may be a source of this variability. The present pilot study evaluated two calibration protocols in different experiments, including static weight and dynamic gait assessment. Results of this pilot study suggested that calibration *pressure* (i.e. force applied over a specific area) may be more important than calibration weight.

In the clinical study we assessed two manufacturer recommended calibration protocols, human and phantom step calibration, performed by three different operators. Results of this clinical study showed that both calibration protocols were highly repeatable and highly reproducible. Although the results obtained with both calibration protocols were statistically different, they were linearly and strongly correlated, making it possible to be directly compared by applying a correction factor. The use of different operators when calibrating a pressure walkway did not influence the results.

In summary, this Master project contributes detailed and meaningful information about the effect of calibration on pressure sensitive walkway results. Based on these results, a specific calibration protocol cannot be recommended, but based on personal experience the use of a phantom during the calibration process may help stability and therefore improve the calibration process overall.

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Author’s declaration

I, Javier Rincon Alvarez, declare that the work in this thesis is original, and was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification. Replication of images or figures from the authors previous publications has been done with approval of copyright from the relevant sources.

Abbreviations

PSW: Pressure sensitive walkway

CCL: Cranial cruciate ligament

GRF: Ground reaction force

PVF: Peak vertical force

VI: Vertical impulse

PP: Peak pressure

BW: Body weight

FL: Forelimb

HL: Hindlimb

Kg: Kilograms

SD: Standard deviation

HS: Human step

PS: Phantom step

CV: Coefficient of variation

ICC: Intraclass correlation coefficient

1. INTRODUCTION

Amongst the most common reasons for companion animals to attend primary veterinary care are musculoskeletal disorders including osteoarthritis, which present with lameness to the veterinary practitioner as the main complaint (O′Neill *et al.*, 2014; Anderson *et al.*, 2018; Summers *et al.*, 2019). Localisation and description of lameness is one of the biggest challenges when assessing a dog's gait. Subjective evaluation of the dog´s gait has been used for many years and it is still the main form of evaluation of gait in a clinical environment. However, the ability to perceive subtle changes and details of the gait in dogs can be challenging. Several studies have shown that subjective evaluation of lameness in dogs varies greatly between observers and either does not correlate, or only correlates in severe lameness, with objective force plate analysis (Evans, Horstman and Conzemius, 2005; Quinn *et al.*, 2007; Waxman *et al.*, 2008).

Objective measures to evaluate gait have evolved greatly over the past 50 years, helping to better understand locomotion (Gillette and Angle, 2008). In order to allow comparison between subjective lameness assessment, visual analogue lameness scales have been validated(Waxman *et al.*, 2008). However, objective gait assessment remains the gold standard(Quinn *et al.*, 2007). Kinematic and kinetic assessment have been broadly used to objectively evaluate the gait in companion animals and although they focus on different aspects of the gait, which will be thoroughly described later. Kinetic assessment remains the option of choice in a clinical setting based on its simpler set up (McLaughlin, 2001). Force plate analysis is still considered the gold standard to assess the gait in dogs. However, pressure sensitive walkways (PSW) are gaining popularity due to their portability, ease of use and possibility of measurement of pressure and force distribution within a paw (Besancon *et al.*, 2003, 2004; Gillette and Angle, 2008; Torres, 2018). These are currently commonly used in a clinical setting in veterinary hospitals and universities all over the world.

This Masters project consists of an extensive literature review of gait analysis with particular interest in PSW, leading to a clinical investigation in the use of a PSW. The first part of the clinical investigation focuses on the understanding of the functioning and calibration of the walkway, leading to a second clinical investigation which aims to investigate and compare the repeatability and reproducibility of two calibration protocols for a PSW.

1.1. Gait and gait cycle

Before any description of gait analysis, some general gait terminology and basic principles of gait must be discussed. Gait is characterized by coordinated and repetitive movements of the limbs and feet (DeCamp, 1997). Gaits are usually divided in to symmetrical and asymmetrical gaits. Walk, trot and pace are symmetrical gaits, which are characterised by the movement of the limbs on one side being repeated by the limbs on the contralateral side, with intervals between foot falls nearly evenly spaced. For example, during trot, the right forelimb and left hindlimb (diagonally located) will give support followed by the contralateral diagonally located limbs. In asymmetric gaits such as the gallop, the limb movement patterns on one side are not repeated on the contralateral side, and footfalls are unevenly spaced. As such, asymmetric gaits are more difficult to interpret and uncommon in gait analysis studies (Nunamaker and Blauner, 1985; Torres, 2018).

A full gait cycle consists of two phases:

Stance phase: defined as the period in which the foot hits the ground and remains on it.

Swing phase: defined as the period in which the foot is not touching the ground.

Together, the stance and swing phase of one foot define one stride (Nunamaker and Blauner, 1985).

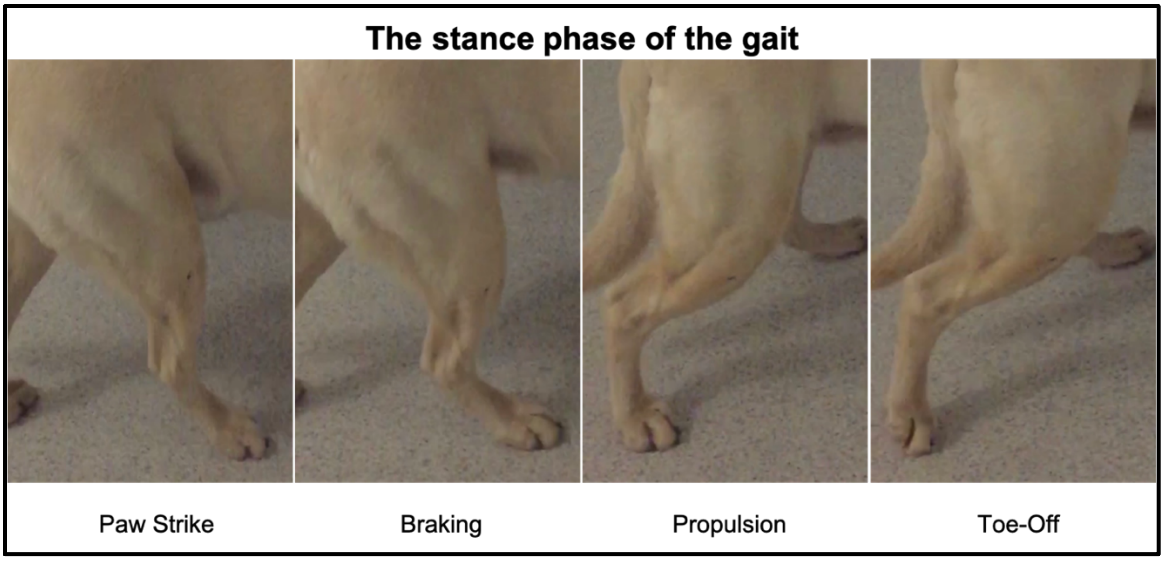


Figure 2.1. Different stages of the stance phase of the gait

The stance phase can be divided into initial paw strike, braking, propulsion and toe off (figure 2.1). Initial paw strike is the first contact of the paw with the ground. Braking happens at the beginning of the stance phase and reduces the forward momentum.

Propulsion occurs when the limb pushes off the ground, increasing the forward momentum. Toe off refers to the paw leaving the ground and indicates the end of the stance phase of the gait. In normal dogs, the forelimb provides more braking than propulsion and the hindlimb provides a greater propulsion than braking (Budsberg, Verstraete and Soutas-Little, 1987).

1.2. Gait analysis

Analysis of gait can involve the study of the spatiotemporal aspects of movement (kinematics) or the forces (kinetics).

1.2.1. Kinematic analysis:

Kinematics is the study of the movement of objects, measuring the position of the body in space, velocities, accelerations and joint angles.

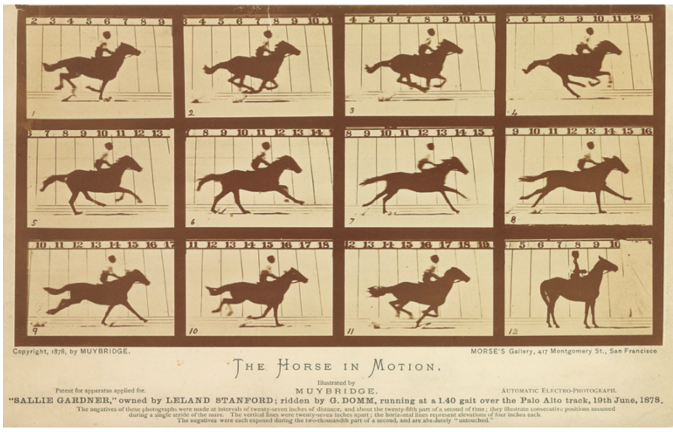


Figure 3.1. The horse in motion. Muybridge. Photo taken from 100photos.time.com

The first kinematic studies began over 100 years ago with Muybridge (1878). He set up an electrically triggered group of cameras to take a sequence of photographs to capture the gallop of a horse (figure 3.1). His aim was to answer the question of whether a horse would ever have all four limbs off the ground when galloping. Muybridge showed that indeed, at certain stages during gallop, horses have all four limbs off the ground. The success of this work led Muybridge to analyse the gait of several other species, such as cats, dogs, monkeys, elephants, camels and raccoons, as well as humans, setting the basis for the 'science' of gait analysis. However, kinematic gait analysis was impractical and difficult to introduce to a clinical setting until the development of photographic recording, making possible the introduction of kinematics to the modern gait laboratory in the 1980's (DeCamp, 1997).

Modern kinematic gait analysis systems (e.g. Qualisys) utilise four to six cameras that record the position of markers located over specific anatomical points of the animal as the animal moves through a defined space. There are two main types of markers used in kinematics to track motion: reflective markers or pulsed light emitting diodes (LEDs). These markers are attached to the skin over specific anatomical landmarks, and the cameras capture the movement of these reflective targets. However, correlation between the markers and the underlying bone structures is not completely accurate, as the movement of the skin and deformation of soft tissues overlying the bone structures has an influence on the marker positions, especially in the moving subject (Kim *et al.*, 2011; Schwencke *et al.*, 2012). Human research studies have shown that markers applied directly to bone via intracortical Hoffman pins (2.5 mm diameter), were more accurate and eliminated the error created by skin markers (Cappozzo *et al.*, 1996; Reinschmidt *et al.*, 1997; Westblad *et al.*, 2002). However, this is not applicable in clinical use in veterinary patients for obvious welfare and ethical issues. The cameras are synchronised and connected to a computer, with specific software used to process the information acquired from the markers (DeCamp, 1997; McLaughlin, 2001; Gillette and Angle, 2008). This technology can gather a great amount of spatiotemporal information, including subject velocity, the segmental velocities of each portion of the limb, stride length and frequency, joint angles (flexion and extension data), angular velocities, and temporal data. However, the equipment is expensive, and difficult to use to a clinical setting (McLaughlin, 2001) - as previously noted, kinematic studies are generally performed in a gait laboratory.

Initial kinematic studies were carried out in healthy dogs to determine normal gait. Hottinger *et al* (1996) evaluated the walk of healthy large breed dogs with a combination of kinematic and kinetic analysis. The results showed minimal motion of the forelimb joints during the stance phase. However, during the swing phase, there was a rapid flexion followed by rapid extension of the elbow and carpal joints, and mainly extension followed by a short period of flexion of the shoulder joint. The hindlimb appeared to have a greater degree of joint movement for all three joints throughout the stance phase, but mainly flexion followed by extension of all three joints during the swing phase (Hottinger *et al.*, 1996). Other studies evaluating gait at the trot in healthy greyhounds and mixed breed dogs reported similar findings, despite the higher velocity due to the trot gait (DeCamp *et al.*, 1993; Allen *et al.*, 1994). Since then, kinematic gait analysis has been used extensively to evaluate lameness in dogs.

Several specific orthopaedic conditions, such as cranial cruciate ligament disease or hip dysplasia, have been assessed with kinematic gait analysis. DeCamp *et al* (1996) showed that dogs with experimentally induced complete cranial cruciate ligament (CCL) rupture had altered movement of the coxofemoral, femorotibial and tarsal joints. The femorotibial joint remained flexed during the whole stride, whereas prior to CCL transection, there was femorotibial extension at the end of the stance phase. Both the coxofemoral and tarsal joints remained more extended when compared to intact CCL gait. This translated into lower propulsion by the femorotibial joint, with compensation of the coxofemoral and tarsal joints helping to preserve the gait by maintaining foot contact with the ground and limb propulsion. These changes in gait suggest an adaptation to stifle instability and pain (DeCamp *et al.*, 1996). More recent studies evaluating dogs with naturally occurring CCL rupture showed comparable results, with shortening of the stride and reduced range of motion of the femorotibial joint (Sanchez-Bustinduy *et al.*, 2010). A later study performing kinematic evaluation on dogs considered to be predisposed to CCL disease (based on a predictive equation that combined two anatomical angle measurements), found similar results involving reduced flexion of the femorotibial joint. However, this study did not follow the cases long-term, and so whether or not the CCL subsequently ruptured was not recorded. Furthermore, the use of femoral and tibial anatomical angles alone as predictors of predisposition to CCL disease has not been validated yet, and the clinical correlation is not fully known (Ragetly *et al.*, 2012).

Gait in dogs with hip dysplasia has also been evaluated with kinematic assessment showing changes of the movement of the coxofemoral joint, involving more rapid extension of the joint during the stance phase and early flexion in the swing phase. The femorotibial and tarsal joints also appeared to have altered movement, with increased flexion throughout the stance and early parts of the swing phase of stride and slow extension in late phase of the stance phase respectively (Bennett *et al.*, 1996). In a study of German Shepard Dogs with radiographic changes suggestive of hip dysplasia, that were not showing lameness when the study was conducted, similar results were obtained, with rapid extension of the coxofemoral joint during the stance phase of the gait. However, kinematics of the femorotibial and tarsal joints did not show significant difference when compared to the control group in that study or when compared to previous studies (Miqueleto *et al.*, 2013).

To overcome the difficulties described with the "classic" kinematic systems (i.e. gait laboratory required, multiple sensors attached to the dog) which may perhaps not represent the normal walking conditions in dogs, inertial sensors have been developed allowing the assessment of angular velocity, orientation, and accelerations of the joints. These are lightweight, portable, motion tracking devices measure, which give information relative orientation of individual body segments rather than direct position (Duerr *et al.*, 2016). These sensors can detect lameness in trotting dogs, and therefore suggested to be an alternative in kinematic studies (Rhodin *et al.*, 2017). However, further studies to validate these sensors in a clinical scenario are required.

1.2.2. Kinetic analysis:

The science of kinetics involves the study of the forces that occur between the foot and the ground during the stance phase of the gait. These forces, known as the ground reaction forces (GRF), arise as a result of Newton's third law of motion: "To every action, there is an equal and opposite reaction" - the GRF are the forces that the ground exerts on the foot when the foot contacts the ground (Richards, 2008; Torres, 2018). The forces can be measured using specific equipment such as force plates, or pressure sensitive walkways. It is important to understand that kinetics studies, do not give information of the individual joints, such as the range of joint motion, or compensation due to lameness. This represents one of the main differences with kinematic analysis.

1.2.2.1. Ground reaction forces (GRF):

As previously described, the stance phase of the gait is defined as the period in which the foot is in contact with the ground. Three orthogonal ground reaction forces arise during the stance phase of the gait: vertical, cranio-caudal (also known as braking and propulsive) and medio-lateral forces (figure 3.2).

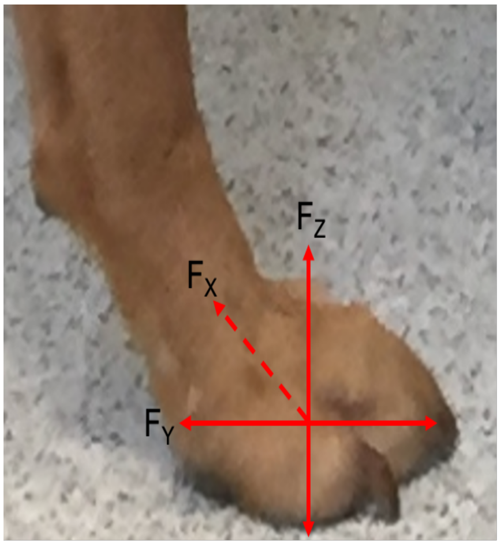


Figure 3.2. Representation of GRF. The vertical force is represented on the Z axis (F_Z), craniocaudal on the Y axis (F_Y) and the mediolateral force represented on the X axis (F_X)

The GRF's are usually represented graphically as force-time curves, as shown below for both the walk and the trot (figure 3.3).

For each force, most studies report both the peak force and the impulse value.

Peak force is the maximum force exerted in a specific direction.

Impulse is derived from the force-time curve and it is the area under the curve for a particular force. This represents the total amount of force during the stance phase of the gait.

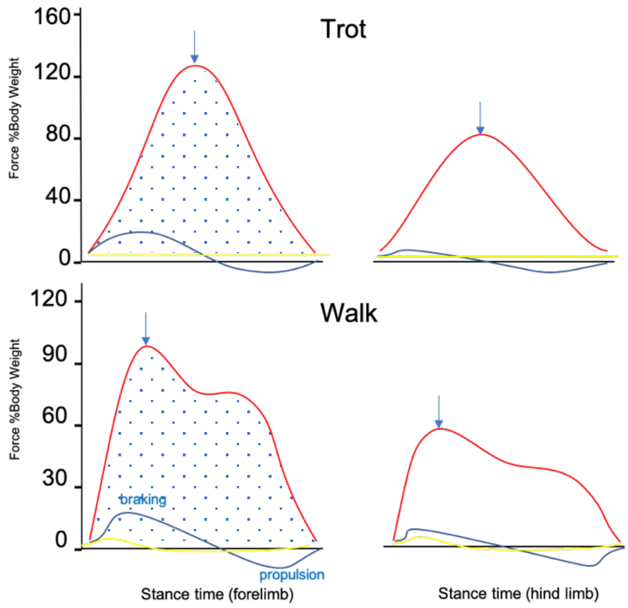


Figure 3.3. Graphical representation of GRF's. The vertical force is represented in red, cranio-caudal force in blue and medio-lateral force in yellow. Blue arrow indicates peak vertical forces (PVF). Dots (area under the curve) represent the vertical impulse (VI)

In gait studies, the vertical and cranio-caudal forces are most commonly reported. Vertical forces are related to the animal's body weight and are the largest of all GRFs. Cranio-caudal forces can be influenced by speed. On the other hand, mediolateral forces are small (representing less than 6% of the body weight) and very variable, both between, and within dogs. Therefore, they are not commonly reported (Budsberg, Verstraete and Soutas-Little, 1987; DeCamp, 1997).

- *Vertical Force.*

  As previously noted, the vertical force (red line) is the largest one and represents the ground reaction force to the subject's bodyweight. Peak vertical force (PVF) is therefore the maximum force perpendicular to the surface of the paw (blue arrow), and vertical impulse (VI) is the area under the vertical force/time curve (dotted area). Both PFV and VI are widely used to evaluate gait, lameness and post-operative outcomes. Studies have shown that both PVF and VI impulse are reduced in animals with lameness - this might be expected as less weight is placed on the painful limb (lower PVF) for a shorter period of time (lower VI) (Budsberg *et al.*, 1988; Budsberg, 2001). Griffon *et al* (1994) surgically induced lameness in the forelimb of healthy Greyhounds and showed that the PVF and VI were reduced not only in the operated limb, but also in the ipsilateral hindlimb. In contrast, the values were increased significantly in the contralateral forelimb and hindlimb. Thus the total amount of force did not change, as the body weight was the same, but was redistributed amongst the other limbs (Griffon, McLaughlin and Roush, 1994). Rumph *et al* (1995) performed a similar study to evaluate force redistribution in surgically induced hindlimb lameness in healthy dogs, showing consistent, and marked increases of PVF on the contralateral hindlimb throughout the 16-week study (Rumph *et al.*, 1995). However, they were unable to show any compensatory loading to the forelimbs as one would have expected based on the results of the study by Griffon *et al* (1994). Therefore, one could expect a greater load redistribution on the ipsilateral and contralateral limbs induced by a forelimb lameness, which is expected, as forelimbs carry a greater proportion of the body weight than hindlimbs, approximately 60 and 40% respectively (Carr, Canapp and Zink, 2015; Kano *et al.*, 2016). One could hypothesise that hindlimb lameness does not require forelimb load redistribution as the 'lower' load can be carried by the contralateral hindlimb. Further studies have used PVF and VI to evaluate lameness and determine the outcome of surgical procedures, showing similar results to the studies previously mentioned (Ballagas *et al.*, 2004; Trumble *et al.*, 2005; Voss *et al.*, 2008; Böddeker *et al.*, 2012; Drüen *et al.*, 2012; Silva, Carmona and Rezende, 2013; Barthélémy *et al.*, 2014; Ferreira *et al.*, 2016; Krotscheck *et al.*, 2016; Rogatko, Baltzer and Tennant, 2016; Sutton *et al.*, 2016).

Other components of the vertical force can be calculated from the graphical representation, such as the rising slope (from zero to the point of maximum force) which represents the rate at which the dog loads the limb. Conversely, the falling slope (from the point of maximum force to the end of the stance phase where the force is zero again) represents the rate at which a dog unloads the limb. These rates are also affected by lameness as Evans *et al* (2005) showed in his study, in which animals with hindlimb lameness have a greater falling slope, indicating a quicker off-loading of the limb. This could be due to pain as the PVF is reached (Evans, Horstman and Conzemius, 2005).

- *Craniocaudal Force*

  The cranio-caudal force (blue line) is the second largest force and can be defined by two periods: the braking and propulsion phases. Braking occurs when the foot/paw hits the ground and is characterised by positive force values. The braking force drops to zero in mid-stance, and then propulsion begins in the second phase of stance, characterised by negative force values, as the foot/paw pushes off and eventually leaves the ground. Peak and impulse values for braking and propulsion forces have also been studied as a measure for detection of lameness, and to determine and compare surgical outcomes. All of these studies showed a reduction of both braking and propulsion of the affected limbs of lame animals, compared to the contralateral normal limb (Budsberg *et al.*, 1988, 1996; Jevens *et al.*, 1996).

1.2.2.2. Force plate:

All three GRFs can be measured using a force plate, which consists of a plate or base containing sensing elements. Traditionally, the plate is imbedded into the ground and is connected to a designated computer to record the different forces. When the subject steps on the plate, its bodyweight (force) causes deflection of the sensing elements which creates a measurable voltage directly proportional to the magnitude of the force. This voltage is translated into a measure of force by a specific software on the designated computer (Anderson and Mann, 1994; McLaughlin, 2001).

The first measurements of force date from the late 19th century. Force plates were rudimental wooden frames on rubber supports. Since then, thanks to technological progress, four main force plate types have been developed: (1) mechanical spring and pointers, (2) linear variable differential transformers, (3) electrical resistance strain gauges and (4) piezoelectric crystals. However, of all these designs, the most commonly used are the resistance strain gauges and piezoelectric crystals (Bonde-Petersen, 1975; Anderson and Mann, 1994).

- *Strain gauge force plate:* Developed in 1975 by Peterson, the strain gauge consists of a piece of wire connected to a metal sensor that changes its electric resistance in proportion to deformation. The strain gauges are connected to an electrical circuit that measures this change on electric resistance in order to measure the force (Anderson and Mann, 1994).
- *Piezoelectric force plate:* Developed by W.P Kistler and C. Sonderegger in 1969, this type of force plate contains certain materials that respond to applied mechanical stress by generating an electrical charge. This is known as the piezoelectric effect. Quartz crystals are an example of a piezoelectric material. In this force plate, the quartz crystals are located on the corners of the plate and creates an electric charge which is proportional to the force applied to the plate (Anderson and Mann, 1994).

It is widely accepted that piezoelectric force plates are more sensitive, being able to measure a greater range of force and being more accurate than the strain gauge force plate (Richards and Thewlis, 2008).

The main limitation of the force plate for quadrupedal gait analysis is that it can be difficult to avoid simultaneous foot contact, generally of the contralateral limb. As a result, a larger number of trials are required to collect 'clean' data, which can introduce variability, such as animal fatigue. Studies have attempted to address this issue by using multiple consecutive force plates in order to collect information from all four limbs on one single pass, with good results, and reduction in the amount of valid passes required (Bertram *et al.*, 1997; Volstad *et al.*, 2017).

Another limitation is the requirement for a highly specialised setup. As previously discussed, the plate is usually embedded in the ground, making this system non-portable. In an attempt to improve the portability of this system, a walkway can be constructed around the force plate level with the surface of the top plate. However, the walkway must not touch the force plate, or it may interfere with data collection. The whole construct is usually covered by a non-slip surface to protect the plate, which might also interfere with the collection of data (Anderson and Mann, 1994; McLaughlin, 2001; Gillette and Angle, 2008).

1.2.2.3. Collecting force plate data:

During the data collection, the dog is walked across the force plate by a handler. The dog must walk in a consistent manner, as any change of position of the head or paws, or direction or speed, can affect the data generated by the force plate. In general, a valid trial is considered as one in which the dog places a forelimb and consecutive ipsilateral hindlimb without placement of the contralateral paws, so no overlap or simultaneous paw placement occurs and the dog crosses the plate at a steady speed (McLaughlin, 2001; Torres, 2018).

A great number of factors can affect the collection of GRFs with a force plate (Jevens *et al.*, 1993; Riggs *et al.*, 1993). The morphology of the dog, velocity which the dog is walked across the force plate, the handler, inter-day variance or variability due to repetition are some of the factors that have been evaluated.

A great amount of work has been done on trying to "normalise" subject morphology, for example by presenting the results as percentage of body weight (%bw) (Budsberg, Verstraete and Soutas-Little, 1987; Voss *et al.*, 2010) which has become a common practice in force plate studies. Subject morphology introduces significant dog-to-dog variation; there is a negative correlation in between PVF and physical size. Budsberg *et al* (1987) showed that at a given speed, larger dogs exerted lower peak forces when results were normalised to the body weight. Larger dogs presented longer stance time and lower PVF, which in other words means that the longer the paw is in contact with the ground the longer the force can be distributed over the musculoskeletal system minimizing the peak loads (Budsberg, Verstraete and Soutas-Little, 1987).

During a force plate evaluation, several trials must be collected. Jevens *et al* (1993) compared the percentage of variance due to dog, handler and repetition. They determined that the coefficient of variance attributable to repetition varied between 28 and 85% and was therefore not negligible. In order to reduce the variability due to repetition, the author estimated the number of repetitions required, creating a statistical design taking into account the number of dogs, number of tests and the suggested variance, which showed an optimal number of repetitions per dog of five. Therefore a minimum of five valid trials are necessary to avoid variation due to repetition (Jevens *et al.*, 1993). Since then, it is common practice in gait analysis studies to use five valid trials.

The variation introduced by handler has been found to be low, approximately 0 to 7% in one study and 8% in other study and can be improved with experience (Jevens *et al.*, 1993; Keebaugh, Redman-Bentley and Griffon, 2015).

The effect of velocity and stance time on GRF's have been the focus of many studies. Riggs *et al* (1993) and McLaughlin *et al* (1994) presented similar results after evaluating sound Greyhounds at the trot, with a positive correlation between the PVF and velocity and a negative correlation between VI and velocity. Similarly, both PVF and VI are correlated to stance time. As the stance time increases (lower velocity as paw stays longer in contact with the ground) the PVF reduces, as previously noted. In contrast, as stance time increases the VI increases as expected as VI is related to the total force during the stance phase of the gait (Riggs *et al.*, 1993; McLaughlin and Roush, 1994). Further studies showed similar results and further explored the correlation between velocity and cranio-caudal forces. They found that braking and propulsion are increased with higher velocities. However, the correlation seems to be weaker (Roush and McLaughlin, 1994; McLaughlin and Roush, 1995; Renberg *et al.*, 1999). Overall, these studies showed that it is important to control velocity when collecting force plate data, as a narrower range of velocities should reduce the variability of GRFs.

The effect of day-to-day variation is controversial. Some studies have shown significant variation in the GFRs in the same group of dogs when data was acquired on different days (Rumph, Steiss and West, 1999; Fanchon and Grandjean, 2009). However other studies have not found any difference between days. Furthermore, most studies investigating outcome or treatment effect only do a one-day gait analysis session, therefore the clinical significance of inter-day variation is arguable.

Finally, an interesting consideration is the assessment of symmetry in normal dogs. Colborne (2008) demonstrated that a single normal Labrador retriever was right hindlimb dominant, and therefore non-symmetrical at a walk. This study introduces the concept of handedness in dogs (Colborne, 2008). However, this has not been reproduced again in later studies, and therefore it is difficult to draw further conclusions.

1.2.2.4. Pressure sensitive walkway:

Pressure sensitive walkways (PSW) contain a variable amount of small pressure sensors distributed throughout their surface. When the subject walks across the PSW, a map of the distribution of pressure underneath the foot/paw is recorded in a computer. This technology is available in different sizes: from each individual foot/pad to long walkways, which can measure pressure all over its surface (Gillette and Angle, 2008). PSWs are commonly used in human gait analysis studies and are increasingly being used in veterinary studies due to their portable nature and the ease of data collection in clinical settings.

PSW do not require a specific set up but to be laid on a flat surface. They are hence considered more portable than force plates. In contrast to force plates, a distinct advantage of the PSW is that useful data can be collected from multiple limbs on a single pass, and individual paws can be evaluated when several feet are on the ground simultaneously (Besancon *et al.*, 2003; Gillette and Angle, 2008; Torres, 2018). This reduces variability introduced through fatigue, as previously mentioned with force plates, which is an important factor when assessing lameness (Beraud, Moreau and Lussier, 2010). Another interesting advantage is the capability of measuring pressure and force distribution across the paw, which has been recently studied in dogs with and without lameness (Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Schwarz *et al.*, 2017; Braun *et al.*, 2019).

Despite these advantages, it is important to understand the differences in data acquisition between PSW and force plates. As noted previously, force plates can measure force in three orthogonal directions (vertical, cranio-caudal and medio-lateral). The main limitation of PSW is that only the vertical forces can be recorded. Moreover, the measure of force (PVF and VI) is not direct as with a force plate, but is calculated from the raw digital pressure once the walkway is calibrated (Gillette and Angle, 2008; Torres, 2018).

Several studies have compared the data obtained with force plates and PSW, showing that although PSWs produce significantly lower GRFs values than force plates, the values are repeatable, making them valid alternatives for gait analysis (Besancon *et al.*, 2003; Lascelles *et al.*, 2006).

1.2.3. Pressure sensitive walkway studies

Due to their ease of use in the clinical environment, PSW's are becoming increasingly popular in veterinary gait analysis. A review of the literature between 2003 and 2019 identified 12 studies where a PSW was used to assess gait in normal dogs. Some studies investigated single breeds including Pitbulls (Souza, Tatarunas and Matera, 2014), Greyhounds (Besancon *et al.*, 2003, 2004), German Shepherds (Souza *et al.*, 2013) and, most commonly, Labradors (Besancon *et al.*, 2004; Agostinho *et al.*, 2015; Schwarz *et al.*, 2017; Assaf *et al.*, 2019). Other studies reported on heterogenous populations of mixed (Fahie *et al.*, 2018), or medium-large breeds (Kim, Kazmierczak and Breur, 2011; Kano *et al.*, 2016) (see table 4.1).

In the studies of mixed populations (Kim, Kazmierczak and Breur, 2011; Kano *et al.*, 2016; Fahie *et al.*, 2018), values for peak vertical force expressed as a percentage of bodyweight (PVF %bw) ranged from 43-74%bw in the forelimbs, and from 27-51%bw in the hindlimbs. Vertical impulse, a measure of total force over time, again expressed as a percentage of bodyweight (VI %bw) was reported to range from 17-24%bw in the forelimbs, and 10-16%bw in the hindlimbs. Even when a single breed was studied (Besancon *et al.*, 2004; Agostinho *et al.*, 2015; Assaf *et al.*, 2019), a wide range of values were reported, ranging from PVF in the forelimbs of 56-95%bw, and in the hindlimbs of 35-65%bw, with VI in forelimbs of 17-35%bw and 10-21%bw. Such variability in results makes it challenging to establish 'normal' ranges, or to make meaningful comparisons between studies.

Variability can be introduced by the calibration process, the process of data collection and analysis, or inherent differences between the subjects themselves. Most studies use standard protocols for data collection and robust statistical analysis, and much work has been done on trying to 'normalise' between subjects. This includes presenting results as %bw, and collecting data within defined ranges for speed and acceleration (Budsberg, Verstraete and Soutas-Little, 1987; Riggs *et al.*, 1993; Besancon *et al.*, 2004; Voss *et al.*, 2010; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Agostinho *et al.*, 2015; Aristizabal Escobar *et al.*, 2017; Schwarz *et al.*, 2017; Braun *et al.*, 2019). In contrast, studies investigating the effect of calibration methods are sparse (Lascelles *et al.*, 2007;

Agostinho *et al.*, 2015). In the majority of PWS studies, the authors report that the 'manufacturer's recommended calibration method' was followed, but do not define it (Besancon *et al.*, 2004; Romans *et al.*, 2004, 2005; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Aristizabal Escobar *et al.*, 2017). A study by Agostinho *et al* (2015) reported that differences in calibration methods resulted in statistically significant differences in the vertical forces subsequently measured (Agostinho *et al.*, 2015). However, the repeatability and the reproducibility of the individual calibration methods was not assessed. This is important in the clinical scenario, where patients may be evaluated at different times by different operators.

1.3. Aims and hypothesis

The aim of this Master project was to investigate and compare the repeatability and reproducibility of two standard manufacturer recommended step calibration protocols for a PSW used to collect data from a heterogeneous population of dogs.

We hypothesised that:

1. The GRF values for each dog would be different between different calibration protocols.
2. Both calibration protocols would be repeatable, but only the phantom calibration protocol would be reproducible.

Table 4.1. Recent canine PSW studies.

Study	Breed	PSW	Calibration method / protocol	Results (%BW)
(Souza, Tatarunas and Matera, 2014)	Pitbull	Tekscan	Mentioned – fixed known weight (protocol not described)	PVF: FL=54; HL=33 VI: FL=23; HL=13
(Assaf *et al.*, 2019)	Labrador Retriever	Tekscan	MS (protocol not described)	PVF: FL=57.55; HL=34.65 VI: FL=17; HL=10.15
(Souza *et al.*, 2013)	German Shepard Dog	Tekscan	Mentioned – fixed known weight (protocol not described)	PVF: FL=55.11; HL=31.7 VI: FL=30; HL=19
(Besancon *et al.*, 2003)	Greyhounds	Tekscan	MS (protocol not described)	PVF: FL=58.18; HL=42.05 VI: FL=25.90; HL=18.59
(Kim, Kazmierczak and Breur, 2011)	Heterogenous medium-large breeds	Tekscan	MS (protocol not described)	PVF: FL= 43.26; HL=26.89 VI: FL=16.57; HL=10.10
(Kano *et al.*, 2016)	Heterogenous medium-large breeds	Tekscan	MS (protocol not described)	PVF: FL=74.45; HL=50.67 VI: FL=24.51; HL=15.63
(Aristizabal Escobar *et al.*, 2017)	English Bulldog	Tekscan	Mentioned – fixed known weight (protocol not described)	PVF: FL=38.6; HL=20.85 VI: FL=8.7; HL=4.7
(Agostinho *et al.*, 2015)	Labrador Retriever	Tekscan	Ten different calibration protocols created and compared	Great variability of results amongst calibration protocols. PVF: FL= (56-95); HL= (35-61) VI: FL= (20-35); HL= (12-21)
(Fahie *et al.*, 2018)	Heterogenous small, medium and large breeds	GAITFour	Calibrated by manufacturer once – No need to calibrate afterwards	Walk: 10-25kg →TPI: FL=30; HL=19.5; 25-40kg→TPI: FL=29.5; HL=20.5 Trot: 10-25kg →TPI: FL=30; HL=19; 25-40kg→TPI: FL=28; HL=18
(Carr, Canapp and Zink, 2015)	Border Collies and Labrador Retriever	GAITFour	Calibrated by manufacturer once – No need to calibrate afterwards	Walk: B. Collie →TPI%: FL=25.4; HL=18.3; Labrador R→TPI%: FL=43; HL=28 Trot: B. Collie→TPI%: FL=33; HL=23.5; Labrador R→TPI%: FL=61; HL=37.8
(Schwarz *et al.*, 2017)	Labrador Retriever	Zebris Medical	No mention of calibration protocol	Measurement of PVF and VI in four quadrants of the paw. Results reported in Newtons and Newtons*s.

MS= Manufacture's specifications; FL= Forelimb; HL= Hindlimb; PVF= Peak vertical force as % of body weight; VI= Vertical impulse as % of body weight; PSW=Pressure walkway; TPI%=Total pressure index percentage

2. MATERIALS AND METHODS

For this section of the Master project, I would like to make a special acknowledgment to my colleague Dr Simone Anesi, as he participated heavily in the design and data collection of the pilot study. I believe that part of the data I will describe and discuss in the following sections will also be included in his Master project as we both participated equally in the understanding of the functioning of the PSW.

2.1. Animals

This Master project was approved by the Ethics Committee of the School of Veterinary Medicine, University of Glasgow. Fifteen staff-owned dogs were enrolled in the study: all were skeletally mature and normal on general clinical and orthopaedic examination, which was performed prior to the data acquisition. General clinical examination was undertaken to assess the animal's cardiovascular system, including mucous membranes, heart rate and pulse rate, and thoracic auscultation to evaluate lung sounds and to exclude the presence of a heart murmur. Temperature was not taken to avoid inducing stress on the dogs prior to data collection. It was also considered unnecessary for the purpose of this study. Orthopaedic examination included a subjective assessment of lameness (presence vs absence) by walking the dogs in the same corridor where the data would be collected, and evaluating each joint for any signs of pain, crepitus, swelling, reduced range of motion or laxity. All general physical and orthopaedic examinations were performed by second (Simone Anesi) - or third (Javier Rincon Alvarez)-year surgery residents. Exclusion criteria were as follows: any systemic illness, history of lameness or orthopaedic surgery within the last 6 months, obesity, cachexia and/or a difficult temperament.

The size of dog enrolled in the study was based on previous publications which had used medium size dogs above 20 kg (Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Agostinho *et al.*, 2015; Schwarz *et al.*, 2017). For this Master project, we changed the weight limit to over 18 kg to include dogs of medium size breeds such as Border Collies or Springer Spaniels (with an ideal body condition score, their weights varied between 18 and 19 kg).

2.2. Equipment

2.2.1. *Specifications and components*

The PSW consisted of a low profile, high-definition system of three sequentially connected plates, with embedded pressure sensors called "sensels" (Strideway HRSW3, Tekscan, South Boston, USA). The sensels produce a raw digital output when they are stimulated by the animal's weight, which is converted by a specific software (Strideway Research, Tekscan, South Boston, USA) into pressure units.

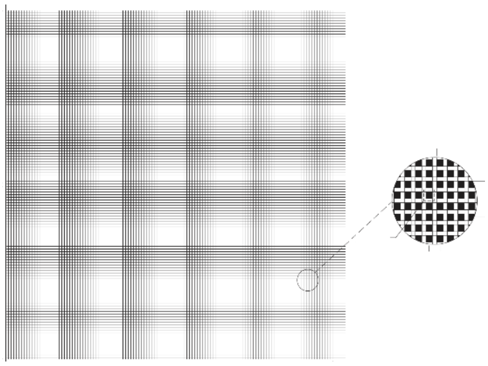


Figure 5.1. Representation of sensel disposition on the pressure plate. Image from Tekscan strideway User Manual

On each plate, the sensels are arranged in columns and rows separated from each other by 1.9 mm of "empty" space creating a honeycomb-like dense panel of sensels. The separation between sensels and the honeycomb-like distribution determines how the PSW will interpret the load applied, and therefore the final pressure output. When the load is applied to the plate's surface by a material that can undergo deformation (i.e. bare foot, foam), part of the load will "sink" into the empty space between sensels. On the other hand, if a material which does not undergo deformation is applied (i.e. sneakers, a stool, plastic), the entire load will lie over the sensel, this will be interpreted differently by the software.

Each plate measured 65.0x91.4x1.5 cm, with a surface of 65.0x26.4x1.5 cm designated to the hardware (i.e. USB connector, power input connectors and microchips; figure 5.2). Therefore, the active sensel surface of each plate was 65.0x65.0x1.5 cm, with a sensel density of 3.88 sensels/cm^2. At either end of the walkway, a tapered non-pressure sensitive plate measuring 65.0x91.4x1.40 cm was added, to create a smooth transition from the ground to the walkway. The final length of the PSW was 325.1 cm with a working length (pressure active length) of 195 cm, containing 48768 sensels.

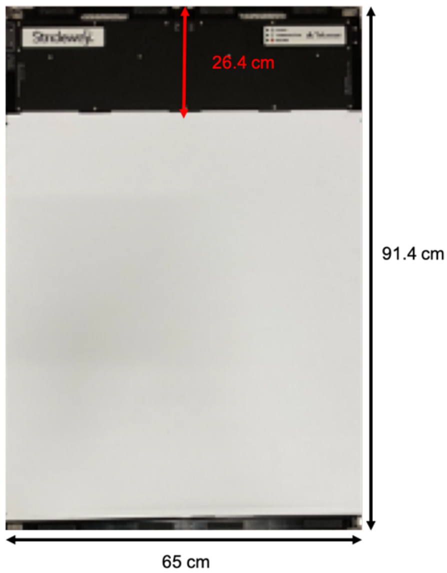


Figure 5.2. Pressure plate. Red arrow: area designated to hardware. Grey area pressure sensitive

The entire walkway was covered by a 0.3 mm thick rubber mat to protect the plates and prevent the dog from slipping. This mat was specifically designed by the manufacturer to fit the walkway and was secured by "Velcro" attachments located all along the plates.

The PSW was connected to a dedicated computer (Lenovo 81 AX, Quarry Bay, Hong Kong) with a specific software (Strideway Research: Tekscan). A high-definition, wide angle video camera (LifeCam Cinema, Microsoft, Washington, USA) was also connected to the computer and synchronized with the PSW by the specific software. The camera was positioned halfway along the PSW's length, approximately 97.5 cm from the first active sensing plate and 60 cm away from the edge of the walkway to capture the length of the walkway (figure 5.3).

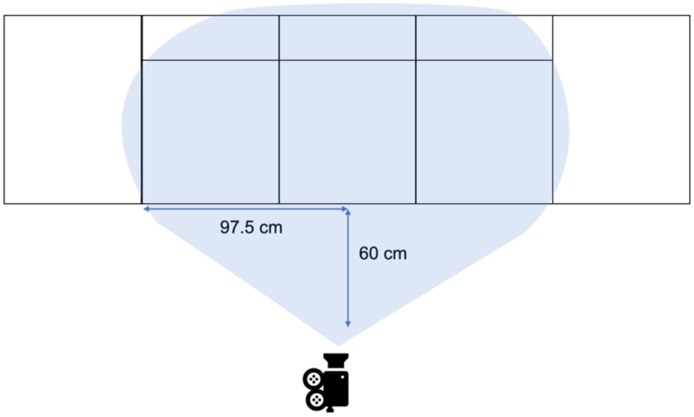


Figure 5.3. Representation of the PSW setting.
Blue: area captured by wide-angle video camera

2.2.2. *PSW set up*

Setting up the PSW followed an eight-step guide as recommended by the manufacturer:

1. The first sensing plate (far right) is placed on a flat surface (floor) and lined up with one of the non-sensitive end plates (figure 5.4). The plates are then attached to each other via two metallic latches.

Figure 5.4. First pressure sensitive plate aligned with the non-pressure sensitive end plate (to left)

2. The next plate is positioned to the left of the first plate. Both plates are lined up and connected by gently pulling on the connector of the plate positioned on the right. This connector will stretch to approximately 6.3 cm, allowing both plates to be connected (figure 5.5). Care should be taken not to overstretch this connector which could result in malfunction of the walkway.

Figure 5.5. Detail of the connection between pressure plates

3. Once connected, the left plate is slid to the right so both plates are flush with each other, attaching them together with the metallic laches (figure 5.6).

Figure 5.6. Detail of pressure plates connected

4. This process is repeated with the third sensing / active plate.
5. The second non-sensitive end plate is then connected to the left end of the walkway, as per step 1 (figure 5.7).

Figure 5.7. PSW formed by three pressure sensitive plates and two (non-pressure sensitive) end plates

6. Once the three plates were in place, the power source was connected to the left tile. Power LED and USB LED displayed a red and green lighting respectively indicating correct connection of the three plates.
7. After 10 seconds, the USB cable was connected to the computer and the Strideway software was initiated.
8. Lastly, the plates were covered with the rollout rubber protective cover (figure 5.8). Particular care was taken not to form any ripples, as this could affect the collection of data.

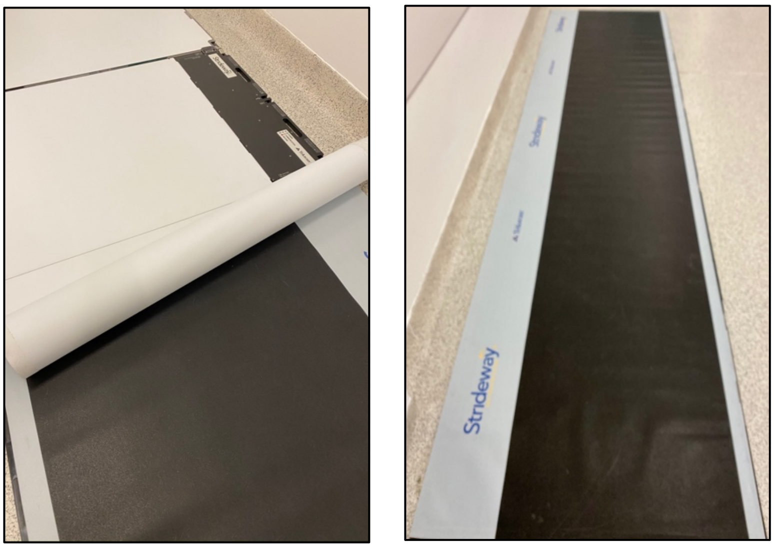


Figure 5.8. PSW covered with a protective rubber cover.

The PSW used for this Master project was the latest version of the Tekscan® system, which is a high definition system with the highest sensel density and total sensel quantity. It is characterised by its portability and versatility, making ideal for a clinical setting as it can be stored in a compact carrier. However, once the PSW is set up for data collection, it is best not to move it until the trial is finished.

There is limited information in the current literature on the optimal environment for collection of gait data using a PSW. It has been suggested to use a designated room with 3 to 4 metres on either side of the walkway (Romans *et al.*, 2004; Lascelles *et al.*, 2006, 2007; Kim, Kazmierczak and Breur, 2011). As a general rule, it is accepted that PSWs should be located in a quiet space, with enough space on either side of the PSW allowing the dog to access it at a constant velocity and to leave it without stopping abruptly.

In this Masters project, the PSW was installed in quiet corridor at the small animal hospital, more than 5 metres long and with at least 2 metres of space on each end of the walkway.

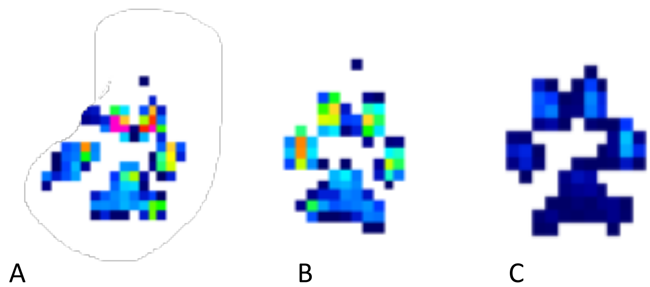
Figure 5.9. Final set up of the PSW

2.3. Sensitivity

The *sensitivity* of the sensels, and therefore the pressure sensitive plates, directly affects the capability of the sensels to convert the raw digital output into pressure units. Three sensitivity settings are available for the PSW: low, medium and high. The sensitivity can be adjusted to allow the sensels to change the level of response to a given digital input. For example: a sensitivity of '1' is where 1 bit equals 1 mmHg with a range of 0 to 255 mmHg. When the sensitivity is adjusted to '2', 1 bit equals 0.5 mmHg resulting in a finer resolution but narrower range (0-127 mmHg). On the other hand, when sensitivity is adjusted to '0.5', 1 bit equals 2 mmHg resulting in a coarser resolution but broader range (0-510 mmHg). This allows the sensels to avoid failing to register, or, conversely becoming saturated, matching accordingly the subject of study. In studies with animals, it is important to adjust the sensitivity to the most appropriate setting for the evaluated animal based on the animal's bodyweight. This process is best explained with the following example:

- A 26 kg dog is walked across the PSW with the sensitivity pre-set at "low". The raw digital output interpreted by the software shows several oversaturated sensels (figure 5.10A). These sensels will not be taken into account once transformed into specific pressure units. This sensitivity is therefore too high for this given animal, and although the definition is very good the range of raw digital output is too narrow.

When the sensitivity setting is adjusted to “high”, the same dog produces a digital output that only reaches the lower aspect of the raw digital output range (figure 5.10C). The sensitivity is therefore too low for this given animal, and although the range of raw digital output is greater, the definition is too low. This will translate into an underestimation during the conversion to pressure units.
Once the sensitivity setting is adjusted to “medium,” the same dog produces a digital output that covers most of the raw digital output range, with no oversaturated sensels (figure 5.10B). Therefore, the “medium” sensitivity setting should be selected for this given dog based on the raw digital output produced on each setting.



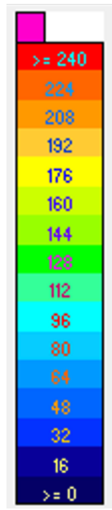


Figure 5.10. Three paw prints of the same dog on three different sensitivity settings. Red and pink represents oversaturation A: low; B: medium; C: high

Thus, sensitivity was selected for each dog prior to collection of any data. The sensitivity settings, low, medium or high were used for small, medium and large animals respectively as per the manufacturer. However, none of the dogs produced a digital output high enough to use the “high” setting, therefore either “low” or “medium” settings were used for all cases.

2.4. Calibration

Calibration is the method by which the raw digital output of the sensel is converted to specific pressure units i.e. KPa, PSI, mmHg. Three different calibration methods are available for the Tekscan walkway, which involve the use of a known weight being applied for different times to each pressure plate. These are described as follows:

2.4.1. *Point calibration*

The known weight used to calibrate the PSW with this method is required to be the subject of study. Before each trial, the subject stands over the pressure plate for at least one second, after which the operator calibrates the plate manually by selecting the calibration tool and once prompted by the system the known weight is finally introduced.

2.4.2. *Step calibration*

The known weight used in this technique can be the patient/animal, another operator, or an inanimate object e.g. weighted disc. In this technique, the software automatically calibrates the plate for 10 seconds once the process is initiated by the operator and after introducing the known weight into the software. For most types of research, this is considered the most accurate technique. As the known weight is not required to be the subject of study, the calibration file can be applied to the data after it has been acquired, providing calibration and data were acquired under the same sensitivity.

2.4.3. *Frame calibration*

This method must be performed after the data has been recorded. The operator identifies a frame within the recorded data that represents the body weight of the subject of study and manually enters the weight into the software to finalise the calibration. For quadrupedal gait analysis, it is somewhat complicated to select a single frame which will represent the body weight of the patient. This is due to the fact that several limbs are placed on the PSW at the same time, but not at the same stage of the gait cycle. This method may be useful when force plate data is available simultaneously, as the force (weight) given by the force plate at a specific instant, can be related accurately to the walkway data. However, this method may be less interesting in a clinical setting.

Step calibration is considered to obtain the most accurate results, as this method takes into account dynamic compensation, which is the compensation of the software to the change in sensel output over time. The manufacturer recommendation for calibration of animal studies is the use of step calibration. However, using the patient to perform the step calibration may not be possible, as it requires the animal to step on to the plate and remain stable for 10 seconds, which in the majority of cases is not possible. When performing step calibration, two main options are therefore suggested to act as a known weight:

- Human: the operator may stand on the plate during the calibration process. The operator will need to keep balanced throughout the process of calibration, therefore stabilisation with a nearby vertical object e.g. a wall, cane, has been proposed (figure 5.11a).
- Phantom: a short three-legged device, consisting of an equilateral triangle with three short legs with a soft 23.6 cm^2 base attached to each leg. This device provides better stability as the short legs are equidistant from the centre of the device. Either a willing operator or known weight-discs can be applied to the base when performing the step calibration (figure 5.11b).

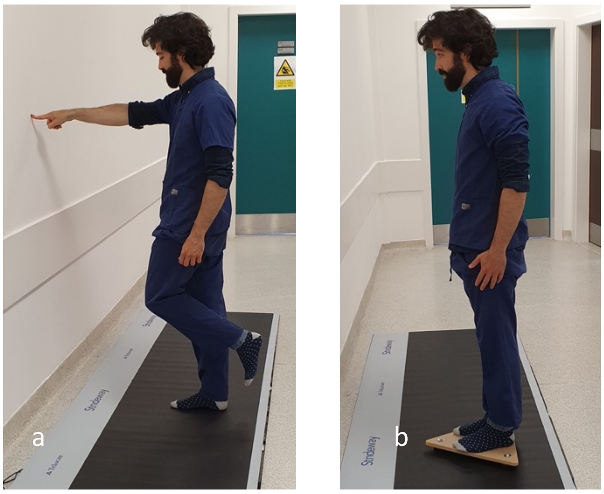


Figure 5.11.

a. representation of step calibration performed by an operator

b. representation of step calibration performed with a phantom by an operator

2.5. <u>Acceleration</u>

Although the PSW has the capability of measuring the acceleration automatically, by calculating the variation in velocity of the paws, this method was inconsistent. In order to be able to calculate the acceleration "manually" (visually), a grid was placed behind the PSW and acceleration was calculated as follows:

- The tip of the nose was followed throughout the video recording of the acquired data and its displacement along the grid was measured at three different points. The lines of the grid were 80 cm apart and therefore the velocity of the nose was calculated between points 1-2 (v^1) and 2-3 (v^2). Acceleration was calculated in an excel spreadsheet by applying the following formula:

$$acceleration = \frac{v^1 - v^2}{time}$$

2.6. Pilot study: investigation of calibration

During the initial stages of the Master project, we faced several challenges related to both the acquiring and interpreting of data from the PSW. The data produced by the PSW was not coherent. It became apparent, after much trial and error, that the calibration process itself had a significant effect on how the data was produced and analysed by the software. Even with guidance from the manufacturer, selection of the optimal calibration method for animal studies is challenging. This therefore became the focus of the first part of the project.

This pilot study aimed to give us an understanding of the "behaviour" of the PSW with different calibration protocols. Following manufacturer recommendations, the step method of calibration was used, as described in the Materials and Methods. Two different protocols were created; for each the sensitivity of the PSW was set at the "medium" setting:

- Protocol 1: Step calibration using a phantom and known weight of 22 kg to match approximately the weight of a medium size dog.
- Protocol 2: Step calibration method using an operator, weighing 67.6 kg.

For each protocol, five experiments were carried out to determine the ability of the PSW to accurately recognise the weight applied:

- Experiment 1: The phantom with the same weight used in protocol 1 (22 kg) was applied to the centre of each pressure plate for approximately 40 to 60 seconds. This experiment was repeated three times on each pressure plate, creating a total

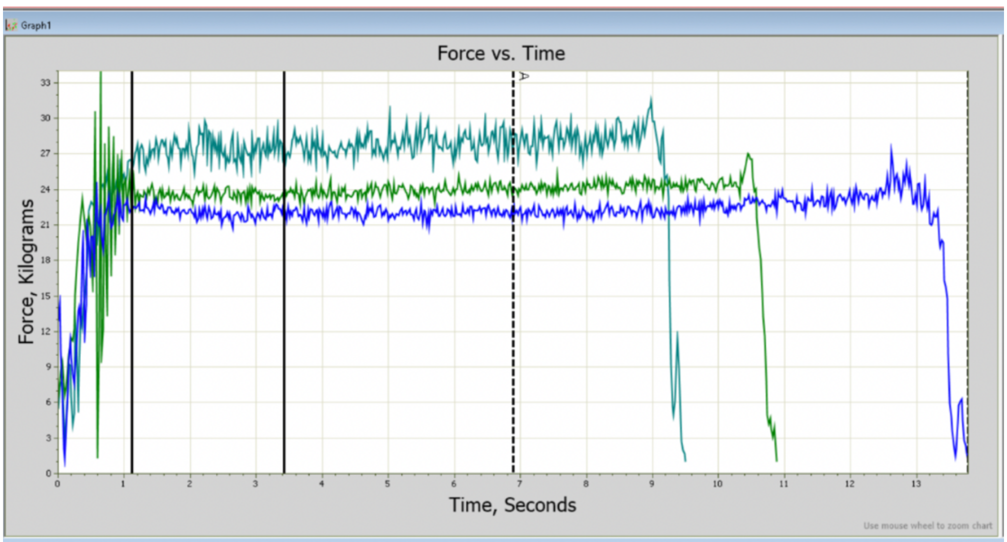


Figure 5.12: Example of force/time curve. Data taken from second 7 (dotted line), where the curve is flat and there is less force variation

of nine repetitions. Force/time curves were automatically generated by the software. After stabilisation of the weight (flattening of the curve), the value of the force was recorded (figure 5.12). The aim of this experiment was to assess accuracy (how close the results were to the real weight) of the PSW to determine static measure of the force (weight).

- Experiment 2: The operator (67.6 kg), who created protocol 2, stood with both feet on the centre of the pressure plate. The operator only wore socks, and this was repeated three times on each pressure plate, obtaining a total of nine repetitions. Force/time curves were created by the software. After stabilisation of the weight (flattening of the curve), the value of the force was recorded as in experiment 1. As in experiment 1, the aim of this experiment was to assess the accuracy of the PSW to determine static measure of the force (weight).
- Experiment 3: The operator (67.6 kg), who created protocol 2, walked across the walkway five times. Values for the PVF and VI, and mean values from the five passes were automatically generated by the software. The aim of this experiment was to assess the accuracy of the PSW for the measure of force dynamically (in movement) applied.
- Experiment 4: A healthy dog (28.4 kg) was walked across the walkway five times. Values for the PVF and VI, and mean values from the five passes were automatically generated by the software. As for experiment 3, the aim of this experiment was to assess the accuracy of the PSW for the measure of force dynamically (in movement) applied.
- Experiment 5: The same dog of experiment 4 (28.4 kg) stood on the centre of the walkway for approximately 60 seconds. Force/time curves were automatically generated by the software. After stabilisation of the weight (flattening of the curve), the value of the force was recorded. As for experiments 1 and 2, the aim of this experiment was to assess the accuracy of the PSW to determine static measure of the force (weight).

For experiments 1, 2 and 5 the mean (+/-SD) of the nine repetitions was calculated and subjectively compared between calibration protocols.

2.7. Clinical study: repeatability and reproducibility of standard calibration method

2.7.1. *Calibration protocols*

Based on manufacturer recommendations for animal studies and pilot study experience, two standard step calibration protocols were created to evaluate their repeatability and reproducibility.

Human Step *(HS):* the operator stood on the centre of each plate on one foot with a sock on for 10 seconds. As recommended by the manufacturer, the operator lightly touched a wall with one finger to improve stability during the calibration process.

Phantom Step *(PS):* the operator stood on a manufacturer-supplied three-legged device with a soft 23.6 cm^2 base attached to each leg, placed on the centre of each plate, for 10 seconds. The weight of the device (1 kg) was added to that of the operator to create each calibration file.

Each protocol was repeated five times by three different operators (weighing (1) 69.5kg (2) 89.2kg and (3) 82 kg, respectively) to create 30 calibration files. Each calibration file was then applied to five runs from each dog, to create 2100 datasets for analysis.

2.7.2. *Data collection*

The pressure walkway was set up to automatically start data collection when a threshold of 500 (raw digital output) was exceeded, and to stop data collection below 200 (raw digital output). Video recording was simultaneously triggered to automatically synchronise with the pressure data. Data was collected over a two-week period in the same corridor, in the same way, by the same handler, with dogs being weighed on the same electronic scale before data collection. Dogs were acclimatised by being walked in a straight line over the walkway several times prior to data collection. An initial trial was recorded to determine the best sensitivity setting for each dog, as previously discussed. This trial was exclusively used to determine the appropriate sensitivity setting and was not included for data analysis. After sensitivity of the walkway was selected, a maximum of 20 trials were collected from each dog to provide five valid trials. Valid trials were identified as those in which the dogs walked in a straight line, without obvious head turning (asymmetry), at a constant velocity between 0.7 - 1.3 m/s with an acceleration no greater than ± 0.1 m/s^2.

All trials were recorded without applying any calibration file, and the raw data were saved for analysis.

2.7.3. *Data analysis*

Each of the 20 recordings for every dog were individually assessed by the author to identify which trials were subjectively valid (i.e. a trial in which the dog walked in a straight line, without obvious head turning, at a constant velocity). Invalid trials were eliminated. Of the remaining trials, symmetry indexes were automatically calculated by the Tekscan software and then evaluated again by the same observer (JRA). As before, trials showing asymmetry were discarded. All remaining trials were considered valid, and from those, five trials were randomly selected for further analysis.

The selected valid trials were then checked for errors in limb allocation - identification of the different limbs was performed automatically by the Tekscan software, but then visually verified by the same operator (JRA), by referring to the synchronized video recording. Limb identification errors were manually corrected.

At this stage, the trials were ready for further analysis to obtain the force, pressure data and spatiotemporal data. In order for this to be performed, each of the 30 calibration files was manually loaded onto each individual trial file, enabling the software to automatically generate the following data: PVF, VI, PP, stride time, stance time, swing time, velocity and acceleration. Data was subsequently analysed in Excel (Excel 2010: Microsoft, Washington, USA).

2.7.4. Statistical Methods

PVF and VI were adjusted by the individual dog's body weights and presented as %bw. Data was determined to be normally distributed by using the normal probability plots and the Shapiro-Wilk W test. As data was normally distributed, numerical variables were presented as the arithmetic mean ±standard deviation (SD), and compared between the groups using a Student's t-test. Only the comparison of vertical forces and temporospatial parameters between breeds was done using a Mann-Whitney U test due to the very small number of dogs being compared.

Repeatability which is defined as the closeness of agreement between test results, obtained with same methodology, on the same conditions, by the same operator, and using the same equipment, was determined using the coefficient of variability (CV), calculated according to the method based on a one-way ANOVA fit to the data containing the repeated measurements made on subjects (Bartlett and Frost, 2008). The following formula was used: CV = M / wSD in which M signified the arithmetic mean and wSD was the within-subject standard deviation.

Reproducibility which is defined as the degree of agreement between the results of experiments conducted by different individuals, with similar instruments. In other words, is defined as the ability to replicate the findings of others under similar conditions, was evaluated using the intraclass correlation coefficient (ICC) (Koo and Li, 2016) calculated using averaged measurements from five calibration subsets. ICC >0.90 signified excellent reproducibility, 0.75-0.90 – good, 0.5-0.75 – moderate and <0.50 – poor.

Agreement between calibration protocols was quantitatively assessed by analyzing 95% limits of agreement (LoA) with their 95% confidence intervals (95% CI) (Martin Bland and Altman, 1986). Measurements averaged for three operators were used. Significance of differences was evaluated using the paired Student's t-test.

Correlation between results obtained in the two calibration protocols was determined using the Pearson's linear correlation coefficient (r). To globally evaluate the influence of all independent variables on pressure measurements (PVF%BW, VI%BW and PP), a mixed linear model (MLM) was developed including three random effects and three fixed effects:

Random variable effects:

- *Dog* (D) to explain variability between dogs from which repeated measurements were obtained
- *Operator* (O) to analyze variability introduced by a random operator performing the study (i.e. reproducibility)
- *Calibration subtype* (C) to analyze variability associated with multiple repetitions (i.e. repeatability).

Fixed variable effects:

- *Calibration protocol* including phantom step (PS) and human step (HS). HS being the reference category. In other words the other category (PS) is compared to the reference (HS)
- *Side* including left (L) and right (R) sides. R being reference category
- *Limbs* including front limbs (FL) and hindlimbs (HL). HL being the reference category

All statistical tests were two-sided with the significance level set at $p < 0.05$.

Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Statistics Inc., Palo Alto, CA, USA), except for ICC and MLM which were developed in IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY, USA).

3. RESULTS

3.1. Pilot study: Investigation of calibration

The results obtained in the pilot study were not subjected to any statistical analysis. The purpose of this part of the Master project, as previously mentioned, was to understand the effect of calibration on PSW data and to familiarise myself with the creation of different calibration protocols, rather than obtaining statistical conclusions. In addition, and for the same reason, a small amount of data was collected precluding any statistical analysis.

3.1.1. *Protocol 1:*

Step calibration of the PSW performed with a phantom and known weight of 22 kg.

Results of the five different experiments performed with this protocol are described as follows:

- Experiment 1: The same weight used in protocol 1 (22 kg) was applied to the centre of each pressure plate for approximately 40 to 60 seconds. The PVF (Kg) results for each plate are represented on table 6.1. Results show a difference from the known weight of 5.9, 3.18 and 16.8% for plates 1, 2 and 3 respectively.

Table 6.1. Results for experiment 1 of protocol 1

	PVF (Kg)			
	Trial 1	Trial 2	Trial 3	Mean (±SD)
Plate 1	22	26	22	23.3 ±2.3
Plate 2	24	22	22	22.7 ±1.2
Plate 3	27	24	26	25.7±1.5

- Experiment 2: The operator (67.6 kg), who created protocol 2, stood with both feet on the centre of the pressure plate. The PVF results for each plate are represented on table 6.2. Results show a difference from the known weight of 15.8%, 9% and 15,8% for plates 1, 2 and 3 respectively.

Table 6.2. Results for experiment 2 of protocol 1

	PVF (Kg)			
	Trial 1	Trial 2	Trial 3	Mean (±SD)
Plate 1	75	79	81	78.3±3.1
Plate 2	76	74	71	73.7±2.5
Plate 3	87	78	70	78.3±8.5

- Experiment 3: The operator (67.6 kg), who created protocol 2, walked across the walkway five times. Results of the five passes are presented in figure 6.1. The average of PVF represents 220% and 209% of the body weight (bw) of the operator for the left and right foot respectively.

Step-Stride Table

Step-Stride Table	PhanT01			PhanT02			PhanT03			PhanT04			PhanT05			Avg		
	TEST Phantom			TEST Phantom			TEST Phantom			TEST Phantom			TEST Phantom			#1, #2, #3, #4, #5		
	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff
Step Time (sec)	0.62	0.58	-0.04	0.60	0.58	-0.02	0.60	0.56	-0.04	0.60	0.54	-0.06	0.58	0.56	-0.02	0.60	0.56	-0.04
Step Length (cm)	57.9	58.4	0.4	59.4	57.3	-2.1	60.5	60.2	-0.2	57.4	56.9	-0.5	55.4	58.6	3.2	58.1	58.3	0.2
Step Velocity (cm/sec)	93.4	100.6	7.2	99.1	98.8	-0.2	100.8	107.6	6.8	95.7	105.3	9.6	95.5	104.6	9.1	96.9	103.4	6.5
Step Length/Leg Length (ratio)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Step Width (cm)	3.2	2.9	-0.3	4.9	5.2	0.3	10.5	10.3	-0.3	5.2	4.7	-0.5	4.5	4.5	0.0	5.7	5.5	-0.2
Stride Time (sec)	n/a	1.20	n/a	n/a	1.18	n/a	n/a	1.16	n/a	n/a	1.14	n/a	n/a	1.14	n/a	n/a	1.16	n/a
Stride Length (cm)	n/a	116.4	n/a	n/a	116.9	n/a	n/a	120.4	n/a	n/a	114.3	n/a	n/a	113.8	n/a	n/a	116.4	n/a
Stride Velocity (cm/sec)	n/a	97.0	n/a	n/a	99.0	n/a	n/a	103.8	n/a	n/a	100.3	n/a	n/a	99.9	n/a	n/a	100.0	n/a
Maximum Force (kg)	148.34	140.81	-7.53	144.03	143.62	-0.40	153.05	142.14	-10.91	149.33	141.60	-7.73	150.78	141.33	-9.45	149.11	141.90	-7.20
FTI (%BW*sec)	302.8	300.6	-2.3	284.3	285.9	1.6	290.9	281.3	-9.6	294.6	270.0	-24.6	284.8	282.4	-2.4	291.5	284.0	-7.5
Maximum Peak Pressure (kPa)	1051	854	-198	1022	771	-251	1056	825	-231	1003	788	-214	974	753	-221	1021	798	-223
FootAngle (degree)	2	13	11	4	14	9	7	11	4	5	13	8	11	10	-1	6	12	6

Figure 6.1. Spatiotemporal values and PVF (Kg), VI (Kg*sec) and peak pressure (PP)(kPa). PVF represented as Maximum force, VI as FTI.

- Experiment 4: A healthy dog (28.4 kg) was walked across the walkway five times. Results of the five passes are presented in figure 6.2. The average results for the PVF represents 101.4 and 53% of the bw for the left fore and hindlimbs respectively and 94 and 52.2% of the bw for right fore and hindlimbs respectively.

Stance-Stride Table

Quadruped Stance-Stride Table	PhanT01				PhanT02				PhanT03				PhanT04				Data001				Avg			
	TEST Phantom				TEST Phantom				TEST Phantom				TEST Phantom								#1, #2, #3, #4, #5			
	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH
Stance Time (sec)	0.56	0.53	0.56	0.55	0.50	0.51	0.51	0.49	0.51	0.49	0.51	0.50	0.47	0.50	0.48	0.47	0.50	0.53	0.55	0.51	0.51	0.51	0.52	0.50
Swing Time (sec)	0.26	0.28	0.29	0.28	0.30	0.28	0.27	0.29	0.27	0.29	0.28	0.30	0.28	0.26	0.26	0.29	0.33	0.30	0.27	0.29	0.29	0.28	0.27	0.29
Stride Time (sec)	0.83	0.83	0.85	0.84	0.80	0.80	0.79	0.79	0.78	0.79	0.78	0.80	0.74	0.74	0.73	0.76	0.85	0.83	0.80	0.83	0.80	0.80	0.79	0.80
Stride Length (cm)	66.3	68.3	65.3	69.1	70.1	72.6	71.6	69.6	72.6	72.6	74.2	71.6	74.7	72.1	75.2	71.6	71.4	68.3	70.1	66.8	71.0	70.8	71.3	69.7
Stride Velocity (cm/sec)	79.9	82.3	76.8	82.2	87.6	90.8	90.7	88.1	93.1	92.0	95.1	89.5	100.9	97.5	103.0	94.2	84.0	82.3	87.6	80.5	89.1	89.0	90.6	86.9
Stride Acceleration 1-2 (cm/sec2)	-3.2	10.6	8.6	8.6	n/a	n/a	-11.6	7.8	0.0	-6.1	n/a	n/a	n/a	n/a	-9.5	-11.5	-8.8	-23.3	-16.2	-4.6	-4.0	-6.3	-7.2	0.1
Maximum Force (kg)	27.64	15.18	25.56	15.09	29.98	15.89	25.55	14.79	27.94	14.66	28.09	15.73	29.81	15.94	26.11	14.22	28.74	13.75	28.17	14.38	28.82	15.08	26.69	14.84
FTI (kg*sec)	11.07	6.00	9.96	6.17	10.47	6.42	9.45	5.28	10.12	5.66	10.26	6.01	10.42	6.02	8.83	5.26	11.02	5.27	10.78	5.77	10.62	5.87	9.85	5.70
Maximum Peak Pressure (kPa)	611	292	403	338	735	299	411	326	648	273	524	320	672	281	428	329	633	253	409	305	660	280	435	324

Figure 6.2. Spatiotemporal values and PVF (Kg), VI (Kg*sec) and peak pressure (PP)(kPa). PVF represented as Maximum force, VI as FTI.

- Experiment 5: The same dog of experiment 4 (28.4 kg) stood on the centre of the walkway for approximately 60 seconds. PVF values approximately 39 kg, representing a difference of 37% from the dog's weight.

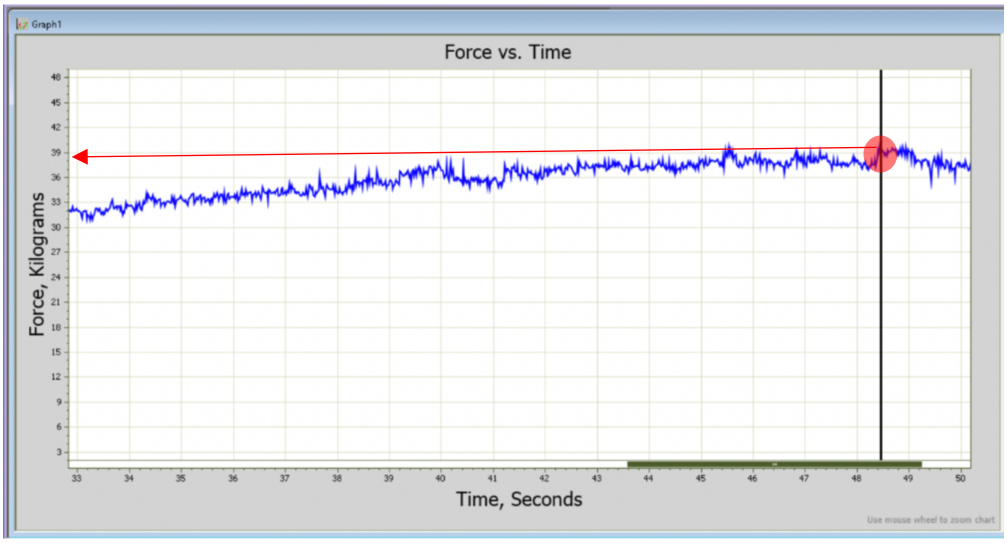


Figure 6.3. Force/time curve. Data obtained after a period of stabilisation of 48 seconds once flattening and less fluctuation was noted.

3.1.2. *Protocol 2:*

Step calibration method using an operator, weighing 67.6 kg. Results of the five different experiments performed with this protocol are described as follows:

- Experiment 1: The same weight used in protocol 1 (22 kg) was applied to the centre of each pressure plate for approximately 10 to 15 seconds. The PVF (Kg) results for each plate are represented on table 6.3. Results show a difference from the known weight of 4.5, 16.8 and 21.3% for plates 1, 2 and 3 respectively

Table 6.3. Results for experiment 1 of protocol 2

	PVF (Kg)			
	Trial 1	Trial 2	Trial 3	Mean (±SD)
Plate 1	23	24	22	23.0±1.0
Plate 2	25	28	24	25.7±2.1
Plate 3	27	28	25	26.7±1.5

- Experiment 2: The operator (67.6 kg), who created protocol 2, stood with both feet on the centre of the pressure plate. The PVF results for each plate are represented on table 6.4. Results show a difference from the known weight of 20.5, 27.5 and 17.6% for plates 1, 2 and 3 respectively

Table 6.4. Results for experiment 2 of protocol 2

	PVF (Kg)			
	Trial 1	Trial 2	Trial 3	Mean (±SD)
Plate 1	55	54	52	53.7±1.5
Plate 2	49	50	48	49.0±1.0
Plate 3	59	52	56	55.7±3.5

- Experiment 3: The operator (67.6 kg), who created protocol 2, walked across the walkway five times. Results of the five passes are presented in figure 6.4. The average of PVF represents 150.6 and 152% of the bw of the operator for the left and right foot respectively.

Step-Stride Table

Step-Stride Table	PhanT01			PhanT02			PhanT03			PhanT04			PhanT05			Avg		
	TEST Phantom			TEST Phantom			TEST Phantom			TEST Phantom			TEST Phantom			#1, #2, #3, #4, #5		
	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff	Left	Right	R-L Diff
Step Time (sec)	0.56	0.56	0.00	0.54	0.54	0.00	0.54	0.56	0.02	0.58	0.56	-0.02	0.58	0.56	-0.02	0.56	0.56	-0.00
Step Length (cm)	54.9	56.9	2.0	59.6	58.9	-0.7	59.3	58.4	-0.9	56.4	58.5	2.1	59.4	60.4	1.0	57.9	58.6	0.7
Step Velocity (cm/sec)	98.0	101.6	3.6	110.4	109.1	-1.2	109.8	104.3	-5.5	97.2	104.4	7.2	102.5	107.9	5.4	103.6	105.5	1.9
Step Length/Leg Length (ratio)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Step Width (cm)	2.3	1.5	-0.8	7.6	8.4	0.7	9.0	9.0	0.0	10.6	10.3	-0.2	1.5	1.7	0.1	6.2	6.2	-0.0
Stride Time (sec)	1.12	n/a	n/a	1.08	n/a	n/a	1.10	n/a	n/a	n/a	1.14	n/a	n/a	1.14	n/a	1.10	1.14	n/a
Stride Length (cm)	111.8	n/a	n/a	118.1	n/a	n/a	117.9	n/a	n/a	n/a	115.5	n/a	n/a	119.9	n/a	115.9	117.7	n/a
Stride Velocity (cm/sec)	99.8	n/a	n/a	109.3	n/a	n/a	107.2	n/a	n/a	n/a	101.3	n/a	n/a	105.2	n/a	105.4	103.2	n/a
Maximum Force (kg)	101.14	95.33	-5.81	104.69	104.83	0.14	108.22	102.22	-6.00	98.77	107.28	8.51	96.31	104.40	8.08	101.83	102.81	0.99
FTI (%BW*sec)	214.6	198.2	-16.4	203.6	194.9	-8.7	208.4	196.1	-12.3	193.0	203.6	10.6	195.8	200.5	4.7	203.1	198.7	-4.4
Maximum Peak Pressure (kPa)	643	708	65	706	675	-30	755	549	-207	736	636	-100	726	684	-43	713	650	-63
FootAngle (degree)	7	9	2	0	12	12	6	6	-0	2	13	12	5	13	8	4	11	7

Figure 6.4. Spatiotemporal values and PVF (Kg), VI (Kg*sec) and peak pressure (PP)(kPa). PVF represented as Maximum force, VI as FTI.

- Experiment 4: A healthy dog (28.4 kg) was walked across the walkway five times. Results of the five passes are presented in figure 6.5. The average results for the PVF represents 75.1 and 37.3% of the bw for the left fore and hindlimbs respectively and 69.4 and 37% of the bw for right fore and hindlimbs respectively

Stance-Stride Table

Quadruped Stance-Stride Table	PhanT01 TEST Phantom				PhanT02 TEST Phantom				PhanT03 TEST Phantom				PhanT04 TEST Phantom				PhanT05 TEST Phantom				Avg #1, #2, #3, #4, #5			
	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH	LF	LH	RF	RH
Stance Time (sec)	0.53	0.54	0.56	0.55	0.55	0.54	0.56	0.55	0.57	0.55	0.55	0.55	0.57	0.54	0.55	0.55	0.57	0.58	0.57	0.55	0.56	0.55	0.56	0.55
Swing Time (sec)	0.29	0.28	0.30	0.28	0.29	0.29	0.28	0.30	0.28	0.28	0.29	0.30	0.27	0.28	0.30	0.31	0.29	0.30	0.29	0.31	0.28	0.29	0.29	0.30
Stride Time (sec)	0.84	0.84	0.84	0.82	0.84	0.85	0.82	0.86	0.85	0.85	0.84	0.84	0.84	0.84	0.86	0.85	0.88	0.88	0.85	0.88	0.85	0.85	0.84	0.85
Stride Length (cm)	66.8	66.3	68.1	62.0	70.4	69.6	71.6	66.5	69.3	69.6	68.8	68.3	69.9	70.6	69.6	68.3	68.8	69.1	67.6	68.6	69.0	69.0	69.1	66.8
Stride Velocity (cm/sec)	79.5	78.9	81.0	75.6	83.8	81.9	87.4	77.4	81.6	81.9	81.9	81.3	83.2	84.1	80.9	80.4	78.2	78.5	79.5	77.9	81.2	81.0	82.1	78.5
Stride Acceleration 1-2 (cm/sec2)	-13.4	16.6	n/a	n/a	-6.8	-5.0	n/a	n/a	1.6	-0.9	6.5	-3.6	-0.7	-4.3	n/a	1.5	-3.3	n/a	-2.2	-0.1	-4.5	1.6	2.2	-0.7
Maximum Force (kg)	20.49	10.14	19.31	10.96	21.68	10.29	20.44	11.03	21.30	11.49	19.85	10.98	21.22	10.60	20.31	9.81	22.03	10.94	18.73	9.84	21.34	10.69	19.73	10.52
FTI (kg*sec)	7.75	4.39	7.62	4.40	8.41	4.35	7.71	4.30	8.67	4.79	7.63	4.25	8.38	4.53	7.67	3.97	8.93	4.70	7.46	3.93	8.43	4.55	7.62	4.17
Maximum Peak Pressure (kPa)	460	195	266	247	480	186	297	248	444	194	329	228	443	198	349	212	476	228	288	216	461	200	306	230

Figure 6.5. Spatiotemporal values and PVF (Kg), VI (Kg*sec) and peak pressure (PP)(kPa). PVF represented as Maximum force, VI as FTI.

- Experiment 5: The same dog of experiment 4 (28.4 kg) stood on the centre of the walkway for approximately 60 seconds. PVF values approximately 26 kg, representing a difference of 2.4% from the dog's weight.

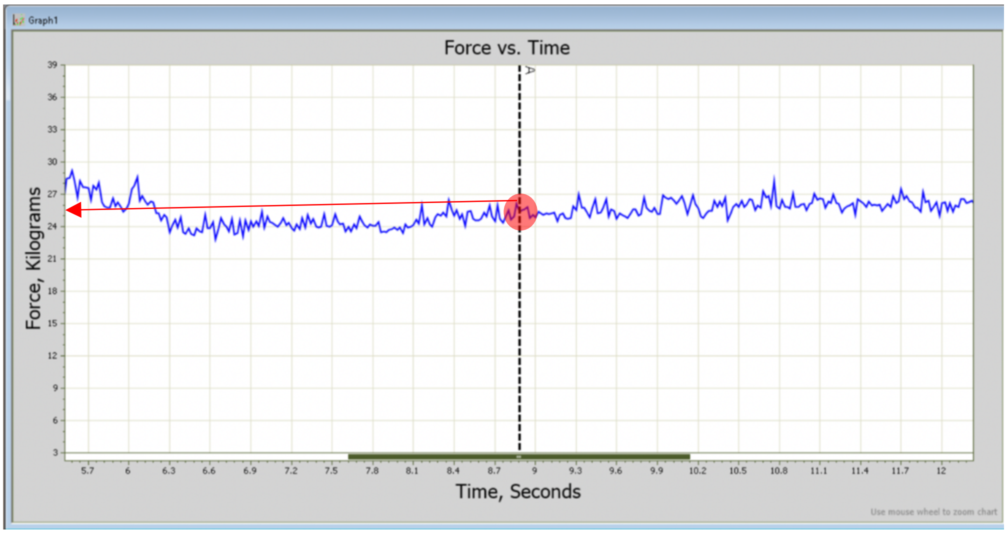


Figure 6.6. Force/time curve. Data obtained after a period of stabilisation. Data obtained on second 9

Drawing final conclusions from the pilot study was challenging. However, it was clear that calibration with a phantom seemed to produce results closer to the previously reported in the literature and therefore expected in our study. However, the question of the weight used for calibration remained unclear based on these results and it was evaluated further on the clinical study.

3.2. Clinical study: repeatability and reproducibility of standard calibration method

A total of 15 dogs were enrolled in the clinical study, but one dog was subsequently excluded due to development of cranial cruciate injury 48 hours after data collection. Of the 14 dogs remaining, five were Labrador retrievers, four were Border Collies, two were crossbreeds and there was one Dalmatian, Springer Spaniel and Huntaway. Seven dogs were male, seven were female and all dogs were neutered. The mean age (± SD) was 4.0 ± 2.14 years (range 1.5 - 10.1), weight was 24.1 ± 3.9 Kg (range 18.0 - 31.4); neither age nor bodyweight differed significantly between males and females.

Temporospatial variables were unaffected by the calibration protocol. Table 6.5 shows the mean values for the 14 dogs for the temporospatial variables.

Table 6.5. Temporospatial variables: mean ±standard deviation (SD)

	Stance Time (s)	Swing Time (s)	Stride Time (s)	Stride Velocity (m/s)
Forelimb	0.49 ± 0.06	0.29 ± 0.03	0.78 ± 0.09	0.98 ± 0.13
Hindlimb	0.46 ± 0.06	0.32 ± 0.03	0.78 ± 0.09	0.78 ± 0.09

s=second; m/s=meter per second

Table 6.6 shows the results obtained from the mixed linear model, which enabled statistical comparison of the following variables: dog, side, front vs hind, calibration protocol, operator and repetition in a single statistical model. No significant differences between left and right limbs were identified for any values, confirming symmetry. There are significant differences between front and hind however, with front limbs having higher values for all measured variables ($p<0.001$).

Table 6.6. Results of the mixed linear model.

			PVF%bw [%]			VI%bw [%]			PP [kPa]		
			Estimate of the model[a]	Stat test	p-value	Estimate of the model[a]	Stat test	p-value	Estimate of the model[a]	Stat test	p-value
Variables fitted as fixed effects	Intercept		35.3±1.6	-	-	11.2±0.9	-	-	223.9±15	-	-
Variables fitted as fixed effects	Calibration	PS[b]	0	-	-	0	-	-	0	-	-
Variables fitted as fixed effects	Calibration	HS	10.3±0.2 (9.9, 10.7)	45.9	<0.001	3.6±0.1 (3.4, 3.7)	43.4	<0.001	58.3±1.5 (55.5, 61.2)	39.6	<0.001
Variables fitted as fixed effects	Side	R[b]	0	-	-	0	-	-	0	-	-
Variables fitted as fixed effects	Side	L	0.4±0.2 (-0.04, 0.8)	1.7	0.08	0.1±0.08 (-0.02, 0.30)	1.7	0.96	-0.4±1.5 (-3.3, 2.5)	0.3	0.8
Variables fitted as fixed effects	Body part	H[b]	0	-	-	0	-	-	0	-	-
Variables fitted as fixed effects	Body part	F	29.1±0.2 (28.6, 29.5)	129.7	<0.001	11.9±0.1 (11.8, 12.1)	145.4	<0.001	119.9±1.5 (117, 122.7)	81.4	<0.001
Variables fitted as random effects	Dog		24.2±9.5 (11.2, 52.5)	2.5	0.011	8.9±3.5 (4.1, 19.2)	2.5	0.011	3059±120 (1415.4, 6611)	2.5	0.011
Variables fitted as random effects	Operator		2.7±2.7 (0.4, 19.7)	0.9	0.324	0.3±0.3 (0.05, 2.4)	1.0	0.325	83.1±84.7 (11.3, 613.1)	1.0	0.327
Variables fitted as random effects	Repetition calibration		0.04±0.1 (0.001, 1.4)	0.5	0.584	0.004±0.1 (<0.001, 0.41)	0.4	0.681	0.6±2.3 (<0.001, 1716)	0.2	0.807
Variables fitted as random effects	Residual (error)		21.1±0.7 (19.73, 22.6)	28.7	<0.001	2.8±0.10 (2.65, 3.04)	28.8	<0.001	909.7±31 (849.78, 973.76)	28.8	<0.001

a: regression coefficient (±SE), and CI (confidence interval) of 95% for variables; b: reference category. p<0.05 indicates statistical significance (values in red)

Results from the mixed linear model (table 6.6) showed no statistical difference for the variables “repetition” and “operator,” confirming the good repeatability and reproducibility. Results obtained from analysis of the coefficient of variability (CV) showed, as with the mixed linear model, that results were highly repeatable (CV from 0.9% - 2.4%) for all operators.

Table 6.7 shows the results obtained from the intraclass correlation coefficient (ICC) test, showing excellent reproducibility (ICC > 0.90) for all variables.

Table 6.7. Intraclass correlation coefficient for PVF%bw, VI%bw and PP for both calibration protocols

	PVF%bw		**VI%bw**		**PP (KPa)**	
	Phantom step	Human step	Phantom step	Human step	Phantom step	Human step
LF	0.906 (0.222, 0.979)	0.918 (0.362, 0.981)	0.961 (0.701, 0.997)	0.965 (0.625, 0.992)	0.976 (0.575, 0.995)	0.980 (0.765, 0.995)
RF	0.938 (0.311, 0.987)	0.945 (0.472, 0.987)	0.97 (0.508, 0.994)	0.972 (0.681, 0.994)	0.978 (0.595, 0.995)	0.982 (0.781, 0.996)
LH	0.919 (0.258, 0.982)	0.934 (0.427, 0.984)	0.941 (0.325, 0.987)	0.945 (0.500, 0.987)	0.949 (0.363, 0.989)	0.955 (0.570, 0.990)
RH	0.933 (0.304, 0.985)	0.940 (0.440, 0.995)	0.955 (0.398, 0.990)	0.955 (0.556, 0.989)	0.963 (0.456, 0.992)	0.966 (0.629, 0.992)

ICC value with CI of 95%

Values for PVF, VI and PP for fore and hindlimbs are represented in table 6.8 a and b. As seen in table 5, body weight distribution (% bw) remained unchanged in either calibration protocol with approximately 30% of the bodyweight on each forelimb and 20% on each hindlimb.

Tables 6.8 a and b. Comparison of mean ± SD PVF%bw, VI%bw and PP in (a) fore and (b) hindlimbs for each calibration protocol, and operator.

Table 6.8a		Phantom step			Human step		
	Limb	Operator			Operator		
		1	2	3	1	2	3
PVF %bw±SD	LF	65.34±6.73	61.25±6.34	63.05±6.33	78.47±8.31	74.14±7.38	75.98±7.88
	RF	65.40±8.16	61.42±7.9	63.08±7.65	78.71±10.47	74.34±9.18	76.20±9.79
VI %bw±SD	LF	23.46±3.84	22.00±3.64	22.64±3.65	28.19±4.85	26.61±4.29	27.29±4.54
	RF	23.43±4.31	22.00±4.13	22.59±4.07	28.20±5.47	26.62 ±4.84	27.29±5.12
PP (KPa) ±SD	LF	347.45 ±73.22	325.81 ±69.33	334.52 ±69.13	415.60 ±90.56	393.58 ±81.08	403.62 ±85.97
	RF	351.93 ±75.63	330.72 ±72.52	338.74 ±71.20	421.57 ±94.48	399.53 ±84.9	409.65 ±89.37
%bw distribution	LF	31.5	31.5	31.5	31.5	31.5	31.5
	RF	31.6	31.6	31.6	31.6	31.6	31.6

Table 6.8b		Phantom step			Human step		
	Limb	Operator			Operator		
		1	2	3	1	2	3
PVF %bw±SD	LH	38.62±4.28	36.22±4.01	37.23±4.12	46.25±5.04	43.83±4.85	44.83±4.95
	RH	37.67±4.59	35.35±4.34	36.37±4.36	45.29±5.48	42.81±5.22	43.81±5.32
VI %bw±SD	LH	12.36±1.58	11.60±1.52	11.93±1.50	14.89±2.05	14.05±1.78	14.40±1.89
	RH	12.10±1.79	11.36±1.71	11.69±1.69	14.62±2.33	13.76±2.00	14.11±2.14
PP (KPa) ±SD	LH	238.80 ±33.01	224.31 ±31.71	230.31 ±30.96	287.17 ±42.47	271.63 ±36.81	278.45 ±39.44
	RH	234.43 ±38.23	220.32 ±36.81	226.09 ±35.87	282.39 ±48.89	266.68 ±42.88	273.75 ±45.52
%bw distribution	LH	18.6	18.6	18.6	18.6	18.6	18.6
	RH	18.2	18.2	18.2	18.2	18.2	18.2

Phantom step calibration resulted in significantly lower values than human step calibration for each of the variables ($p<0.001$) as noted in table 6.9:

- PVF%bw was between 8-19% lower in front legs, and between 5-11% lower in hind legs.
- VI%bw was between 2-8% lower in front legs, and between 1-4% lower in hind legs.
- PP was between 21-117 kPa lower in front legs and between 20-79 kPa lower in hind legs.

However, the results of both protocols were strongly linearly correlated ($r>0.99$ for all measurements)

Table 6.9. Agreement between the two calibration protocols.

		Difference between Phantom step and Human step calibration		Paired Student's t-test	Pearson's linear correlation coefficient	
		Mean (SD)	CI 95%	p-value	r	CI 95%
PVF%bw	LF	-13.35 (1.60)	-14.28, -12.43	<0.001	0.993	0.977, 0.998
	LH	-7.70 (0.92)	-8.23, -7.17	<0.001	0.994	0.981, 0.998
	RF	-13.51 (1.95)	-14.64, -12.39	<0.001	0.998	0.993, 0.999
	RH	-7.75 (0.99)	-8.33, -7.18	<0.001	0.993	0.977, 0.998
VI%bw	LF	-4.81 (0.96)	-5.36, -4.25	<0.001	0.999	0.997, 1.000
	LH	-2.57 (0.44)	-2.82, -2.31	<0.001	0.998	0.993, 0.999
	RF	-4.84 (1.03)	-5.43, -4.25	<0.001	0.999	0.997, 1.000
	RH	-2.56 (0.50)	-2.85, -2.27	<0.001	0.999	0.997, 1.000
PP [kPa]	LF	-69.03 (16.12)	-78.34, -59.72	<0.001	0.999	0.997, 1.000
	LH	-48.66 (9.09)	-53.90, -43.41	<0.001	0.997	0.990, 0.999
	RF	-69.93 (16.90)	-79.69, 60.17	<0.001	0.999	0.997, 1.000
	RH	-48.76 (10.05)	-54.56, -42.96	<0.001	0.998	0.993, 0.999

p<0.05 shows statistical significance

Two breeds were overrepresented in our population, Labrador retrievers and Border collies. Statistical comparison of their gait was performed to determine if there was a breed related gait pattern, which could influence the results. Results showed no significant differences between breeds, except for the PP of the left forelimb as seen in table 6.10a.

Tables 6.10 a and b. Comparison of gait parameters between Labrador retriever and Border collies

Table 6.10a	Forelimb					
	Left			Right		
	Labrador retrievers (n=5)	Border collies (n=4)	p	Labrador retrievers (n=5)	Border collies (n=4)	p
PVF%bw	63.12 (61.68-68.02)	61.00 (55.72-62.50)	0.063	63.50 (56.32-69.30)	59.94 (55.66-61.90)	0.286
VI%bw	26.38 (20.12-28.42)	20.98 (19.92-22.60)	0.063	26.74 (20.04-28.8)	20.66 (19.08-22.62)	0.190
PP (Kpa)	363 (294-429)	265 (250-340)	0.032	370 (272-421)	284 (250-354)	0.190
Stance Time	0.56 (0.43-0.58)	0.48 (0.45-0.52)	0.190	0.55 (0.43-0.59)	0.48 (0.45-0.52)	0.286
Swing Time	0.31 (0.25-0.33)	0.27 (0.25-0.30)	0.413	0.31 (0.26-0.32)	0.28 (0.26-0.29)	0.413
Stride Time	0.87 (0.68-0.91)	0.76 (0.70-0.80)	0.413	0.87 (0.68-0.90)	0.76 (0.71-0.81)	0.286
Stride Velocity	0.89 (0.82-1.07)	1.01 (0.93-1.14)	0.111	0.89 (0.81-1.07)	1.01 (0.92-1.14)	0.111

Table 6.10b	Hindlimb					
	Left			Right		
	Labrador retrievers (n=5)	Border collies (n=4)	p	Labrador retrievers (n=5)	Border collies (n=4)	p
PVF%bw	34.28 (32.74-46.50)	39.14 (34.80-43.86)	0.413	37.80 (28.32-41.80)	36.20 (31.66-43.44)	0.730
VI%bw	12.28 (11.16-14.72)	12.14 (10.82-12.44)	0.730	12.76 (10.08-14.84)	11.18 (11.12-11.78)	0.730
PP (Kpa)	244.4 (210-291)	216.1 (207-230)	0.190	226 (194-312)	206 (193-217)	0.111
Stance Time	0.51 (0.39-0.54)	0.45 (0.40-0.47)	0.190	0.52 (0.40-0.56)	0.44 (0.41-0.48)	0.286
Swing Time	0.34 (0.30-0.35)	0.32 (0.28-0.34)	0.413	0.33 (0.28-0.35)	0.31 (0.29-0.34)	0.413
Stride Time	0.87 (0.69-0.90)	0.75 (0.70-0.80)	0.190	0.87 (0.69-0.90)	0.76 (0.71-0.81)	0.286
Stride Velocity	0.89 (0.80-1.07)	1.01 (0.92-1.14)	0.111	0.89 (0.81-1.06)	1.00 (0.91-1.12)	0.111

$p<0.05$ shows statistical significance (red)

4. DISCUSSION

The original idea of the Master project was to use the pressure mat to study the gait of dogs with lameness as a result of cranial cruciate ligament disease. Specifically, we were interested in understanding the effect of concurrent meniscal damage on the pattern of lameness, and whether the pressure mat could be used to differentiate between dogs with and without concurrent meniscal damage. Much literature currently exists on the study of canine gait using force plates, and increasingly, the pressure sensitive walkway (PSW) is used as an alternative. However, soon after starting the literature review, it became apparent that although values reported in force plate studies seemed consistent, those reported in PSW studies varied greatly. Colleagues and authors of previous PSW studies were contacted, and while the significant inter-study variability was acknowledged, a consensus could not be reached on the reason(s) for it.

When our new PSW was delivered, learning to use the system for the first time involved a steep learning curve. Despite assurances over a period of months from the company (Tekscan) that supplied the mat that our seemingly widely erroneous results could be explained, we were eventually able to prove to them that the system was faulty and they replaced it for us. Working with our new replacement system, the results were much more realistic, as they were closer to those previously reported but still seemed inconsistent. The focus of the Master project therefore changed completely, to firstly investigating the PSW-related factors that could cause such variability not only between studies but within our own study, and then explore this further by using the system to collect and analyse the gait of normal dogs.

4.1. Investigating and controlling factors affecting variability:

In the introduction of this Master project, several factors that introduce variability into kinetic studies have been outlined. Most of these factors are common to both force plates and PSW and have been the focus of initial kinetic gait analysis studies. Although these studies have set the basis for the identification and control of these factors in later investigations, some of these were re-visited and questioned in the initial stages of this Master project.

4.1.1. *General factors affecting variability:*

- Dog-related: size / morphology / weight

Dog size has a marked influence on gait analysis studies. For a given speed, smaller dogs produced higher PFV and VI (normalised to bw) than larger dogs (Budsberg, Verstraete and Soutas-Little, 1987). As larger dogs have longer strides, a particular speed could represent the walking speed for large dogs and trotting speed for smaller dogs, as speed can be considered as the length of the stride by the frequency of the strides. Therefore, depending on the dog's size, the same speed will determine the type of gait (walk vs trot) and it should be adjusted to the dog's size to maintain similar gaits so that valid comparisons can be made. This makes standardisation studies challenging. In agreement with Budsberg *et al* (1987), Kim *et al* (2011) showed that small dogs have significantly shorter stance and swing phases and overall shorter gait cycles than large dogs when moving at a walking pace (Kim, Kazmierczak and Breur, 2011).

For the clinical study in this Master project, dog size was controlled as all dogs were considered medium size (18 to 35 kg). This weight was selected based on many other PSW studies (Evans, Gordon and Conzemius, 2003; Besancon *et al.*, 2004; Lascelles *et al.*, 2006; Kim, Kazmierczak and Breur, 2011; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Agostinho *et al.*, 2015; Kano *et al.*, 2016; Aristizabal Escobar *et al.*, 2017; Assaf *et al.*, 2019). which had used dogs over 20 kg. However, from our experience and based on our selected population, the weight of medium size breeds such us a Border Collie with a perfect body condition score, was between 18 and 20 kg. Therefore, we considered 18 kg the lower weight limit for the clinical study.

The effect of breed on the results of kinetic analysis is a subject of controversy. Multiple studies assessing treatment outcomes have evaluated heterogenous groups of dogs and found no statistical differences attributed to the breed (Nelson *et al.*, 2013; Vassalo *et al.*, 2015; Krotscheck *et al.*, 2016; Rogatko, Baltzer and Tennant, 2016). Furthermore, studies assessing the gait of clinically normal heterogenous groups of dogs i.e. large, medium and small size dogs, found no significant differences within each of the groups (Kano *et al.*, 2016; Fahie *et al.*, 2018). Conversely, other studies have shown significant differences in gait between breeds (Bertram *et al.*, 2000; Mölsä, Hielm-Björkman and Laitinen-Vapaavuori, 2010; Voss *et al.*, 2010; Carr, Canapp and Zink, 2015). Bertram *et al* (2000) noted differences between Labrador Retrievers and Greyhounds, showing that the latter, at a trot, used fewer and longer strides than the Labradors (Bertram *et al.*, 2000). Carr *et al* (2015) showed that the total pressure index (sum of peak pressure for a paw during contact with the mat, in relation to the total amount of pressure of all limbs) was lower for Border Collies compared to Labrador Retrievers. Furthermore, Border Collies spent a significantly shorter proportion of both the walking and trotting gait cycles with their thoracic and pelvic limbs in contact with the ground than did the Labrador Retrievers (Carr, Canapp and Zink, 2015). It is important to note that these studies showed differences related to the spatiotemporal parameters of the gait i.e. stride length or stance phase, but direct comparison of ground reaction forces was not performed. A single study has shown significant differences in ground reaction forces between Rottweilers and Labrador Retrievers, with significantly lower PVF in thoracic limbs and significantly higher vertical impulses in thoracic and pelvic limbs in Rottweilers compared to Labradors (Mölsä, Hielm-Björkman and Laitinen-Vapaavuori, 2010). In the same study, when effect of body weight, functional limb length and relative velocity (velocity relative to limb length) were removed from the statistical model, no significant differences were noted between breeds, showing that indeed, morphology of the dog (breed related) introduced variability. In our study, no significant differences were found in a heterogenous group of dogs in the PVF, VI or PP that could be attributed to the heterogenicity of the sample. Furthermore, when the two overrepresented breeds of this study sample, Border Collies and Labrador Retrievers, were compared separately to each other, neither the GRFs, PP or spatiotemporal parameters were significantly different, with the exception of PP for the left forelimb (p=0.032). These results suggest that breed does not affect PSW results. However, it is important when considering these results to take into account the small sample size which could have induced a type II statistical error.

- <u>Trial related: measure and control of velocity and acceleration</u>

As previously discussed in the introduction section, controlling the velocity and acceleration is important during gait analysis, as such parameters can introduce variability in the data (McLaughlin and Roush, 1994, 1995; Roush and McLaughlin, 1994; Renberg *et al.*, 1999). An early study by Riggs *et al* (1993) on force plates showed that increments of velocity of 0.6 m/s would introduce significant variability in the GRF results (Riggs *et al.*, 1993). In a subsequent study, McLaughlin *et al* (1994) determined that even increments in velocity of less than 0.6 m/s could introduce variability in the GRF results in dogs, and concluded that control of acceleration is crucial, as a positive acceleration will increase propulsive forces and decrease braking forces (McLaughlin and Roush, 1994). Most force plate studies measure the average speed of the subject using start/interrupt timing devices, which do not take into account variation in speed (acceleration) during the trial. Although acceleration can be calculated, as it can be in PSW studies, force plate data are acquired at one specific time of the dog's pass: when the subject steps on the force plate. However, in PSW studies, data is obtained throughout the whole pass of the dog (i.e. data for the same paw is measured several times during the same pass) as the device is longer. Therefore, maintaining a steady velocity and controlled acceleration becomes crucial in PSW studies. Interestingly, no description of the methodology of measurement of acceleration is described in the majority of current studies using PSW.

In this Master project, the designated PSW software had the capacity to obtain acceleration automatically. However, as this was calculated based on paw placement, it was necessary that placement of the same paw was recorded at least three times for the software to be able to calculate the acceleration of that paw. For a medium size breed, such as a Border Collie or small Labrador Retriever, this was possible within the pressure active length (1.95 metres) at a walking speed. In larger breed dogs with longer stride lengths, the pressure active length was not enough to record the same paw placement more than twice, precluding the software from calculating the acceleration automatically. To overcome this problem, a grid was positioned along the side of the walkway (opposite to the video camera), and acceleration was calculated by analysing the video recording, as described in the materials and methods section.

Regarding acceleration reported in current PSW literature, ranges varied from ±0.1 to ±0.5 m/s^2 (Evans, Gordon and Conzemius, 2003; Besancon *et al.*, 2004; Lascelles *et al.*, 2006; Kim, Kazmierczak and Breur, 2011; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Agostinho *et al.*, 2015; Kano *et al.*, 2016; Aristizabal Escobar *et al.*, 2017; Assaf *et al.*, 2019). Although this has not been directly evaluated for a PSW system, based on the results from Riggs *et al* (1993) and McLaughlin *et al* (1994), an acceleration of ±0.5 m/s^2 may introduce variability and therefore in this clinical study an acceleration of ±0.1 m/s^2 was preferred. The effect of acceleration on PSW results warrants further investigation.

4.2. Pressure sensitive walkway-related factors affecting variability: the most challenging aspect and the least understood

In understanding possible sources of variability, it is important to first understand in more depth how the PSW works i.e. how exactly is the weight of the animal converted for a force / area to create a pressure?

As previously described in the materials and methods section, embedded in the pressure plate there are thousands of pressure sensitive sensors called sensels. These sensels are made from plastic material that undergoes elastic deformation (being able to return to the initial shape) when a load/weight is applied. This change in shape produces an electrical charge (difference in voltage) that is translated into a raw digital output by the specific software (Strideway Tekscan), represented as a coloured pixel on the screen. A "map" of coloured pixels is displayed from each stimulated sensel.

Furthermore, it is important to understand the distribution of these sensels as a honeycomb-like structure, as noted in the materials and methods section, and this will have an impact on the interpretation of the weight applied to the sensels. Using the previous example of a bare foot vs sneakers: part of the surface of the bare foot will sink into the "empty space" between the sensels, as the foot adapts to the surface of the PSW. On the other hand, the sneakers are less elastic and will not sink into the "empty space". This will be interpreted differently by the software, as it registers the force exerted on each sensel and calculates the pressure by dividing it by the total area (sensel and empty space). This calculation assumes that the force is evenly distributed over all the surface area. Therefore, materials with less elasticity, such as sneakers, produce different results as force is distributed less uniformly. This raises the question of texture when applying weight to a PSW. Agostinho *et al* (2015) investigated the effect of texture when calibrating a PSW by comparing barefoot, socks and sneakers and determined that each of them had an influence on the results (Agostinho *et al.*, 2015). In the present clinical study, the question of texture was taken into consideration by the use of a phantom: this will be discussed further in this section.

Finally, it is important to remember that the sensels may become oversaturated under high weights. However, this may be controlled by adjusting the sensitivity setting making it possible to adjust the definition of the response of the sensels, allowing them to respond accurately to wide range of weights.

Therefore, to be able to obtain a specific pressure measurement (force divided by surface area) by the software, the sensels must undergo a complicated and critical process of calibration. This calibration process is much more complex than for a force plate, as the latter simply generates an electric charge that is in direct proportion to the load applied to the sensors. A large range of loads can be applied, anywhere on the plate, and the electrical charge is instantly produced and directly converted by the software into units of force.

As discussed above, this process of calibration will be determined by:

- The sensitivity level chosen
- The surface area and texture applied to the sensel
- The calibration file applied to convert the electrical output to pressure/force unit.

4.2.1. *Selection of the appropriate sensitivity*

As previously described, the sensitivity of the sensels directly affects the capability of the sensels to convert raw digital output to pressure units. There are three sensitivity settings available in this PSW (low, medium and high), which are recommended by the manufacturer to be used with low, medium and high subject weights respectively. This concept is counterintuitive, as a low sensitivity setting equates to the highest sensitivity of the sensel. This produces a high-definition digital output, although within a reduced range of raw digital output, as explained in the materials and methods section. It is then understandable why this setting is recommended to be used with low weights, achieving a high-definition response and discouraged to be used with high weights, as the sensel would oversaturate due to the reduced raw digital output range. In animal studies it is therefore simple to select a correct sensitivity setting with extreme weights (i.e. cats vs horses; low and high sensitivity settings being selected respectively). However, selection of the optimal sensitivity level for medium size dogs, as are used in this Masters project, becomes more complex.

Data for medium size dogs, as evaluated in this Master project, can be acquired with all three sensitivity settings. However, only one would produce optimal results. Selection of the optimal sensitivity setting was thoroughly described in the materials and method section. Briefly, the optimal setting will produce a sensel response which utilises as much of the raw digital output range as possible without oversaturating the sensels. Although this process was not standardized, it allowed subjective selection of the most appropriate setting based on the dogs sensel stimulation while walking and was therefore performed on each of the dogs prior to any data collection.

From personal experience, selection of sensitivity for medium size dogs, was not only determined by the animal's weight but also the dog's conformation. Dogs of similar weights, such as a large Border Collie or slim Labrador Retriever would have a different most appropriate sensitivity setting (low and medium respectively). This shows that perhaps dog morphology and conformation have an effect on the sensel stimulation. Labradors in general are more muscled than slim Border Collies and their muscle mass concentrates on the forequarter, whereas in Border Collies it seems to be perhaps more evenly distributed (*Border Collie Breed Standard*, 2020; *Labrador Retriever Breed Standard*, 2020). Although this is a hypothesis, this concept would further support the findings by Carr *et al* (2015), showing that Labradors and Border Collies have indeed a different gait (Carr, Canapp and Zink, 2015). Surprisingly, there is no description of sensitivity selection in the literature, and this could represent one important source of variability of results in PSW studies. This area merits further study, assessing how each individual sensitivity setting affects the PSW results. Based on my experience in this study, the author recommends evaluating each animal individually in all three sensitivity settings, as described in the materials and methods, to select the optimal setting for the individual animal prior to any data collection.

Having selected the appropriate sensitivity, the next calibration challenge was understanding why the PSW did not give a sensible output value relative to the weight of a person standing on it.

4.2.2. *Lack of accuracy in measuring weight distribution of a person standing on the PSW*

During the early stages of training in the use of the PSW, a lack of accuracy for the measure of total weight was identified. This inaccuracy was noted when an operator was standing still on the PSW, which was expected to accurately recognise the weight of the operator. However, generally higher weights (up to 37% higher) were noted. This is in contrast to the force plate system, which can be simply and accurately calibrated by the operator standing on it.

As previously noted, the PSW sensels are made of plastic, which undergo deformation when a weight is applied. The sensels have the capacity to adapt to the weight and return to the initial shape once the weight is no longer applied to them. This feature makes the sensel output not totally linear, with a fast initial response, and a slower response that compensates for the changes in the sensel output over time. This concept is known as 'drift' of the sensels. The drift can be accounted for and controlled by the step calibration method, as the weight is applied up to 10 seconds while calibrating. Other methods of calibration, in which the weight is applied for less time, may not account for this - this is discussed later (section 7.3.1). The software can then interpret the deformation of the sensel to the applied weight. However, during weight distribution assessment, application of constant weight is required over longer times, making the output of the sensel inaccurate. This is likely to be due to a greater sensel deformation than the one that is taken into account by the PSW during calibration.

Although PSW are fundamentally designed to measure pressure during dynamic gait, accuracy for static weight distribution measurement in a PSW has been evaluated, showing encouraging results when compared to a weight distribution platform (Bosscher *et al.*, 2017; Clough *et al.*, 2018). Weight distribution platforms are weighing scales connected in four quadrants over which the subject/animal stands, measuring distribution of the weight on each limb. However, these were experimental studies, with application of static loads mounted on a purposely designed jig, therefore their clinical application needs yet to be determined. In our pilot study, static weight was evaluated in three of the experiments (experiments 1, 2 and 5) by applying different weights: static weight on a phantom, human standing on the plate and a dog standing on the plate, for approximately 60 seconds. Some of the results obtained were encouraging, with errors between 2.4 and 9%, and comparable with those of previous studies of 3 to 4% (Clough *et al.*, 2018). However, the overall results showed a great variability with results between 2.4 to 37% of error. These results were markedly influenced by the different calibration protocols, making it unfortunately very challenging to determine which calibration protocol was best when measuring static loads. As previously mentioned, the results in the pilot study were not subject to statistical analysis, as the aim of the pilot study was to familiarise ourselves with the challenges of calibration and to determine the best calibration protocol to use in the clinical study. Therefore, acquisition of more data and statistical analysis might have shown less inaccuracies, as shown in the current literature (Bosscher *et al.*, 2017; Clough *et al.*, 2018).

- *Weight distribution in clinical studies*

  Several studies have shown weight redistribution due to osteoarthritis on the forelimbs (Bockstahler *et al.*, 2009; Braun *et al.*, 2019), with transfer of the weight to the contralateral and ipsilateral fore and hindlimb respectively. In addition, Kirpensteijn *et al* (2000) demonstrated that in animals undergoing limb amputation there was a marked weight redistribution to the contralateral limb, as well as change in the centre of gravity of the body (Kirpensteijn *et al.*, 2000). All these studies justify the need to further investigate the use of a PSW to assess static loads and weight distribution, as with this technology more data can be collected with fewer trials. This is crucial in animals with osteoarthritis as there is evidence that exercise i.e. excessive gait analysis trials, can introduce variability in gait trials due to fatigue (Beraud, Moreau and Lussier, 2010).

Having investigated the dynamic vs static response of the sensors during calibration, the next challenge was in selecting the most appropriate and accurate method of calibrating the PSW for canine gait studies.

4.2.3. *Selection of a method for calibration in animal studies*

Based on previous studies, different methods of calibration of a PSW will lead to discrepancies in the results (Agostinho *et al.*, 2015). We therefore undertook a pilot study to allow us to understand the effect of subject weight and calibration method on the data acquired with the PSW.

As expected, and in agreement with the current literature, the results of our pilot study showed marked variability in the results of the two calibration protocols, except for experiment 1, in which a known weight of 22 kg attached to a stable phantom device was applied to the pressure plate for approximately 60 seconds. In this experiment, Protocol 1 – step calibration created with a phantom and 22kg weight, gave mean results of PVF of 23.3, 22.7 and 25.7 kg for each of the pressure plates respectively, whereas protocol 2 – step calibration created by a 67.6 kg operator, produced mean PVF of 23, 25.7 and 26.7 kg for each pressure plate respectively. Although, as previously discussed, results of the pilot study were not subjected to statistical analysis. A maximum of a 2 kg difference (9 % of error) was noted. The accuracy of the protocols created was assessed subjectively, showing that protocol 2 seemed to produce more accurate results for the assessment of the walking gait, both for a human operator (experiment 3) and a dog (experiment 4). Protocol 1 produced results of PVF over 200 % of the bw of the operator on each foot at a walk, which represent more than double the reported forces at a walk in humans (Barela *et al.*, 2014). For the walking dog, PVF results were approximately 100 and 50% of the bw for the fore and hindlimb respectively, representing much higher values than previously reported in dogs with PSW. As previously discussed, reported values of PVF for dogs at a walk are variable, between 50-70% of bw for the forelimbs and 25-45% of bw for the hindlimbs (Besancon *et al.*, 2004; Kim, Kazmierczak and Breur, 2011; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Kano *et al.*, 2016; Aristizabal Escobar *et al.*, 2017; Assaf *et al.*, 2019). However, for protocol 2, results for experiment 4 (walking dog) were comparable to those previously reported, with PVF approximately 75% and 37% of bw of fore and hindlimbs respectively (Agostinho *et al.*, 2015; Kano *et al.*, 2016).

These results may be counterintuitive as the weight used in 'protocol 2' was substantially higher than the subject's weight (22 kg), and 'protocol 1' should perhaps be more accurate as the calibration weight was closer to the subject's weight. Previous studies have raised the same question: how closely should the calibration weight mimic the subject's weight (Agostinho *et al.*, 2015)?

Based on these pilot study results, the weight by itself may not be as significant as pressure is (pressure being the force divided by the surface area over which it is distributed), as the latter will determine the extent to which the sensels are stimulated and whether they are overloaded. This is supported by other studies, for example, Lascelles et al (2007), who reported that calibration by a person of approximately 50 kg created pressures in the range of approximately 0.4 kg/cm^2, similar to those of the cats under study (0.3-0.5 kg/cm^2), thus operator weight should not affect the results (Lascelles *et al.*, 2007). Taking this into account, the pressure created by the calibration in protocol 1 and 2 were approximately 0.36 and 1.2 kg/cm^2 respectively. Pressure created by the paw of the dog evaluated in experiment 4 was 1.1 kg/cm^2. This may therefore explain the discrepancies noted with protocol 1 as the pressure of the calibration was lower than those of the subject of study.

The results of this pilot study were in accordance with those of Agostinho *et al* (2015) (Agostinho *et al.*, 2015), as different calibration protocols produced different results. However, based on the results discussed above, we considered that in fact the calibration weight may not be as relevant as the pressure is, and therefore we created the calibration protocols for the clinical study within the range of pressures created by dogs walking.

The next (clinical) stage of the study involved assessing the repeatability and reproducibility of two standard calibration protocols recommended for PSW studies, by applying them to analyse the gait of normal dogs.

4.3. Clinical study: repeatability and reproducibility of standard calibration methods

In the present study, the two calibration protocols, created by three operators, were applied to analyse gait data from 14 normal dogs. The results demonstrated that significantly different values are obtained when different calibration protocols are used on the same raw data, and so we accept our first hypothesis. However, the values derived using each individual calibration protocol were highly repeatable, and highly reproducible between operators ($p<0.001$). We therefore accept our second hypothesis in part.

Despite promising results for repeatability and reproducibility, there are still significant discrepancies between calibration protocols, which makes comparison between PSW studies challenging.

4.3.1. *Variability due to calibration methodology*

The majority of published studies involving pressure sensitive walkways use the Tekscan® system (Besancon *et al.*, 2003, 2004; Romans *et al.*, 2004, 2005; Lascelles *et al.*, 2007; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Agostinho *et al.*, 2015; Stadig and Bergh, 2015; Aristizabal Escobar *et al.*, 2017). However, few studies have investigated the effect of calibration technique on PSW results; a 2015 study by Agostinho *et al* (2015) is the most comprehensive. The latter study used point and step calibration methods, which have been extensively described in the materials and methods section. Briefly both involve applying a known weight to the pressure walkway for a specific length of time. The step calibration method is recommended for animal studies (Tekscan, 2017) where the PSW recording is automatically triggered in response to load, using either a person of known weight (Lascelles *et al.*, 2007), or a short three-legged stool with a centrally loaded weight (a 'phantom') , which is thought to be more stable (Tekscan, 2017). In this study, the authors identified significant differences in PVF and VI when calibration was performed by individuals of different bodyweight, with bare feet versus sneakers (attributed to textural effects) and stepping onto the PSW with one versus two feet (attributed to contact area) (Agostinho *et al.*, 2015). In our study, no difference was found when calibration was performed by operators of different weights. These results were highly reproducible. The effect of weight in the Agostinho *et al* (2015) study may have been due to the different method of loading the sensels. As previously discussed, the drift of the sensels needs to be taken into account when performing the calibration of a PSW. In our study, step calibration was used, where the load is maintained for 10 seconds allowing the software to record the changes in the sensel's output while a constant load is maintained. The drift is therefore taken into account by the software while converting raw data into pressure data. In contrast, when point calibration is used, as in the Agostinho *et al* (2015) study, the load is applied for a shorter period, and the effect of drift (the 'slow response factor') may not be taken into account. The duration of application of load during calibration protocols may therefore be a significant factor that should be standardised in PSW studies.

As previously discussed, the weight by itself may not be significant, but the pressure is. In the present study, the pressure range created by the dogs was 0.8 to 1.69 kg/cm^2, while that of the operators ranged from 0.9 - 1.68 kg/cm^2 for the PS protocol, and 1.2 - 2.02 kg/cm^2 for the HS protocol. It is therefore unlikely that in the present study, as in previous studies, the difference in weight between the operators and the dogs had a significant effect on the results.

The question of texture (i.e. sneakers vs socks vs bare feet), which may play a significant role in the response of the PSW sensors, merits further investigation. Although the phantom has been proposed to reduce the variability introduced as a result of different foot size and texture between operators, previously reported as potential sources of variation (Lascelles *et al.*, 2007; Agostinho *et al.*, 2015), we found no statistically significant evidence to support this, as reproducibility between operators was as high with and without the phantom. Furthermore, the high reproducibility between operators suggests that in contrast to concerns raised in previous studies, minimum variability is introduced by the potential clinical scenario of having different operators collect data at different times. This is an important finding, as in a clinical setting it is unlikely to have a single operator to calibrate the PSW always available, therefore different clinicians could calibrate the PSW without introducing significant variability, making the use of PSW simpler in a clinical setting.
Introduction of the phantom also aims to improve the stability of the operator while creating the calibration file, which is thought to improve the accuracy of the data. The manufacturer recommends using a vertical object e.g. wall or stick, to aid balance while performing step calibration to minimise movement (instability) (Tekscan, 2017). Although both calibration protocols were equally repeatable and reproducible, in the author's experience, the use of the phantom simplified the calibration process, with fewer attempts required to create a valid calibration file.

Although ideally the same calibration protocol should be used between studies, our finding that the calibration protocols produced results that were strongly linearly correlated, raises the possibility of applying a correction factor to facilitate comparisons between studies if the calibration method is adequately described. Further work would be required to evaluate this.

In the second part of this project, gait data was obtained from normal dogs, providing a reference resource for future PSW studies.

4.3.2. *Canine gait data*

Temporospatial parameters were unaffected by calibration protocol, which is expected as these parameters are independent of pressure load and only dependent on time and distance. However PVF, VI and PP differed significantly depending on the calibration protocol, in agreement with previous studies (Lascelles *et al.*, 2007; Agostinho *et al.*, 2015). Significant differences were identified between PVF and VI for forelimbs and hindlimbs in all protocols, as expected, as forelimbs carry approximately 20% more of weight than hindlimbs in normal dogs (60 and 40% bw respectively)(Lascelles *et al.*, 2006; Carr, Canapp and Zink, 2015; Kano *et al.*, 2016). In this study, the Phantom Step protocol produced consistently lower results for all parameters compared to the Human Step protocol: PVF%bw was between 8-19% lower in front legs, and between 5-11% lower in hind legs. VI%bw was between 2- 8% lower in front legs, and between 1-4% lower in hind legs and PP was between 21-117 kPa lower in front legs and between 20-79 kPa lower in hind legs. Despite this, all results were in agreement with those reported in other studies. In the present study we reported results of PVF%bw between 60-75% and 35-46% and VI%bw between 22-28% and 11-14% for fore and hindlimbs respectively, comparable to, for example, PVF %bw ranging from 54-74% and 33-50% and VI %bw ranging from 17-23% and 10-15% for forelimbs and hindlimbs respectively, in Labradors, Greyhounds, German Shepards and heterogenous group of dogs (Besancon *et al.*, 2003, 2004; Souza *et al.*, 2013; Souza, Tatarunas and Matera, 2014; Assaf *et al.*, 2019).

Although convention dictates that forces be expressed as %bw to try to normalise between dogs of different weight (Budsberg, Verstraete and Soutas-Little, 1987; Voss *et al.*, 2010), our data suggests that PVF%bw by itself cannot be used to make direct comparisons when different calibration protocols are used. In agreement with Agostinho *et al* (2015) however, the % body weight distribution (%bwd) remained the same independent of calibration method, with approximately 30%bw on each forelimb and 20%bw on each hindlimb. The %bwd was consistent between PSW studies (Lascelles *et al.*, 2006; Kim, Kazmierczak and Breur, 2011; Souza *et al.*, 2013; Kano *et al.*, 2016; Assaf *et al.*, 2019) making it a potentially more useful measure to compare results between studies. Previous studies have shown that there was no statistical difference in the sensitivity of PVF, VI or %bwd when used to compare limb usage after orthopaedic procedures (Seibert *et al.*, 2012), making the %bwd a valuable measure for assessment of lameness. Furthermore, we previously noted that pathologies such as osteoarthritis (Bockstahler *et al.*, 2009; Braun *et al.*, 2019) and limb amputation (Kirpensteijn *et al.*, 2000) will cause weight redistribution producing changes on the %bwd, hence its interest as a tool for the assessment of gait.

4.3.3. *Study limitations*

Study limitations include the small sample size and the heterogenous population of dogs used. More animals could have been included in the study, which may have shown more difference in gait between breeds e.g. Labrador Retriever vs Border Collie. However for the purpose of assessing repeatability and reproducibility of the two calibration protocols, the current sample size (giving 2100 data sheets for analysis) was sufficient to have strong statistical evidence. Including more animals would have protracted the data analysis, already delayed, due to the difficulties aforementioned with the equipment. Heterogenicity of the sample may have introduced variability which we did not account for. Inclusion of more animals in the study may have helped to determine variability related to breed. However, this remains controversial as several studies have not showed significant breed-related differences.

Calibration files were created on a single day, whereas dog trials were collected over a 2-week period, potentially introducing variability. Rumph *et al* (1999) suggested that inter-day variation of vertical ground reaction forces was not negligible and should be taken into account when comparing data from the same animal. The origin of this variation was suggested to be a combination of biological mechanisms (i.e. stress, willingness to cooperate, hunger) and external factors (i.e. weather, time of the day) (Rumph, Steiss and West, 1999). In this clinical study, data for each dog was collected within a single day, in proposed sources of variability. Even though we acknowledge that the conditions when each of the dogs were walked may not have been the same, reproducing them on different days would have been extremely challenging. Time of the day, data collection when subjects have been starved or fed, and/or limiting stress by reducing the time taken for data collection could have been controlled and should be taken into account for future studies. Lastly, all calibration files were created on a single day- this was purposely done to reduce any environmental factors, such as temperature or humidity, which can influence the functioning of the PSW. However, this is a potential limitation as in a clinical setting, the calibration file may be created on different days, as dogs present at different times and require to be re-evaluated several times. The inter-day variability in generating PSW calibration protocols has not been investigated before and further studies are therefore warranted.

4.3.4. *Conclusions*

Although each calibration protocol yielded different PVF, VI and PP results in a heterogeneous group of dogs, the results were highly repeatable and reproducible for the individual calibration protocols. In addition, the results of both calibration protocols were strongly linearly correlated, potentially facilitating comparisons between different studies. The author recommendation is to evaluate each animal individually in all three sensitivity settings, as described on the materials and methods, to select the optimal setting for the individual animal, prior to any data collection. Although one protocol has not been shown to be superior to the other, in the authors’ experience, the Phantom Step calibration provided a more stable surface making production of the calibration file easier.

This clinical study was presented as a short communication in the ECVS residents forum 2020 and has been published in the Veterinary and Comparative Orthopaedics and Traumatology in October 2020 (see Appendix).

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